SCIENTIFIC POSTER SESSION SCHEDULE

Posters of the accepted abstracts can be viewed in Hall A of McCormick Place, on Tuesday, August 6 and Wednesday, August 7. All posters will be posted from 9:30am until 5:00pm. Presenting authors will be in attendance from 12:30pm until 1:30pm. Please refer to the onsite Abstracts Title Guide for a complete schedule of posters.

Below are the topics and their scheduled times.

TUESDAY, AUGUST 6, POSTER SESSIONS

9:30am – 5:00pm

Biomarkers of Acute Cardiovascular Diseases . . . . . A-001 – A-037 . . . . . S2
Clinical and Diagnostic Immunology . . . . . A-038 – A-125 . . . . . S13
Clinical Translational Science . . . . . A-126 – A-143 . . . . . S41
Proteomics and Metabolomics . . . . . A-144 – A-149 . . . . . S48
Endocrinology . . . . . A-150 – A-225 . . . . . S51
Factors Affecting Test Results . . . . . A-226 – A-273 . . . . . S75
Hematology and Coagulation . . . . . A-274 – A-310 . . . . . S92
Informatics . . . . . A-311 – A-321 . . . . . S103
Animal Clinical Chemistry . . . . . A-323 – A-332 . . . . . S107
Tumor Markers and Cancer Diagnostics . . . . . A-333 – A-387 . . . . . S111
Infectious Disease . . . . . A-389 – A-459 . . . . . S128

WEDNESDAY, AUGUST 7, POSTER SESSIONS

9:30am – 5:00pm

Lipoproteins and Vascular Diseases . . . . . B-001 – B-022 . . . . . S150
Management Sciences and Patient Safety . . . . . B-023 – B-066 . . . . . S158
Mass Spectrometry and Separation Sciences . . . . . B-067 – B-110 . . . . . S172
Molecular Pathology . . . . . B-111 – B-138 . . . . . S186
Nutrition . . . . . B-140 – B-150 . . . . . S196
Pediatric and Maternal Fetal . . . . . B-152 – B-181 . . . . . S200
Critical and Point-of-Care Testing . . . . . B-182 – B-228 . . . . . S211
Proteins/Enzymes . . . . . B-229 – B-259 . . . . . S227
TDM and Toxicology . . . . . B-260 – B-310 . . . . . S238
Technology/Design Development . . . . . B-311 – B-352 . . . . . S255

Author Index S269

Keyword Index S281

Ed. Note: These abstracts have been reproduced without editorial alteration from the materials supplied by the authors. Infelicities of preparation, grammar, spelling, style, syntax and usage are the authors’.

Tuesday, August 6, 2019

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

Biomarkers of Acute Cardiovascular Diseases

A-001

Categorizations and Distributions of Troponin T Results Before and after Switch to High-Sensitivity Troponin T (Roche Elecsys Troponin T Gen 5 STAT)

J. Guarente, J. E. Hollander, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Our institution switched from standard troponin T (TnT, Roche Diagnostics USA) to "high-sensitivity" troponin T (hs-TnT, Roche Diagnostics USA, Elecsys Troponin T Gen 5 STAT) in April 2018. We examined laboratory data before and after this switch to evaluate distributions of results among different results categories (within reference interval (WRI), elevated (E), or critical (C)), and for changes in followup testing among first results among different results categories.

Methods: Data for troponin orders, for a period of 45 days before (PRE) and after (POST) the switch to hs-TnT, were retrieved from the laboratory information system. Summary statistics were examined for distributions of clinical categorization of results: PRE (WRI: <0.01 ng/mL; E: 0.01-0.1 ng/mL; C: >0.1 ng/mL); POST (WRI: <19 ng/L; E: 19-53 ng/L; C: >53 ng/L), and rates of second orders from first orders as a function of clinical categorization of first results. Data were analyzed using Excel spreadsheets.

Results: Dataset PRE was comprised of 6069 results from among 3206 patients (1.89 results/patient). Dataset POST was comprised of 6195 results from among 3004 patients (2.06 results/patient). There were changes in clinical categorizations of first results as follows: WRI: 77%(PRE), 66%(POST); E: 17%(PRE), 21%(POST); C: 6%(PRE), 12%(POST). These data represent an increase (by 11% of the total number of first results) in first results outside of the reference interval, and an increase (by 6% of the total number of first results) in critical first results. For dataset POST, first results in category of WRI included a new subcategory of WRI having quantifiable results (viz., WRI results that were above the lower limit of quantitation (LLOQ), 6 ng/L), comprising 55% of all WRI first results; for dataset PRE, first results WRI were by definition all below LLOQ (0.01 ng/mL). For dataset POST, 66% of first results received at least one follow-up troponin measurement, compared to followups for only 47% of first results for dataset PRE, a change of 19% among the total number of first results. The increase was primarily from followups from among first results in category of WRI. Among categories of first results, percentages receiving followups were as follows: WRI: 37%(PRE), 55%(POST); E: 78%(PRE), 86%(POST); C: 84%(PRE), 89%(POST). For followups among WRI(POST), the majority (71%) were from category of (WRI, >LLOQ).

Conclusions: After adoption of hs-TnT, there was an increase from 23% to 34% in the fraction of first results outside of the reference interval, with an increase from 6% to 12% in the fraction of first results categorized as critical. Among first results for hs-TnT, the majority (55%) were in a new category of (>LLOQ, WRI). There was an increase from 47% to 66% among first results receiving a followup measurement, accounted for primarily by the increase in follow-up tests ordered on first values in category of WRI. Although these data are from only the first 45 days after startup of hs-TnT, the distribution of first results categorizations are likely to be stable, whereas followup rates may well change as institutional experience with hs-TnT increases.

A-005

Study of Serum Ischemia Modified Albumin and Other Conventional Cardiac Markers in Acute Myocardial Infarction within 6 Hours of Onset of Chest Pain

S. Chhetri. *Manipal College of Medical Sciences, Pokhara, Nepal*

Background: The NH_2-terminal of albumin gets altered leading to reduced binding of albumin with cobalt within few minutes of ischemia. This albumin altered by ischemia is called Ischemia modified albumin. Ischemia modified albumin levels can be assayed by spectrophotometric techniques. Previous studies done in other parts of the world have shown IMA to be a promising marker for the diagnosis of MI. However, studies regarding IMA from Nepal are scarce. We conducted this study to compare the diagnostic performance of IMA and other conventional biomarkers in patients with ACS presenting within six hours of onset of chest pain. **Aims and Objectives:** The study was undertaken to evaluate the diagnostic importance of Ischemia modified albumin in people with chest pain of suggestive of Myocardial Infarction presenting early to hospital. This study also aims to compare the diagnostic performance of IMA with conventional cardiac biomarkers. **Materials and Methods:** A hospital based, cross sectional, descriptive, case control study was carried out with 50 cases of chest pain and 50 healthy controls. Study was conducted after obtaining ethical clearance from institutional review committee of the hospital. All participants were enrolled in the study after obtaining informed written consent. Venous blood of cases and controls were processed in department of biochemistry, Manipal Teaching Hospital and quantitative values of IMA and other conventional biomarkers (cTnI, CK-MB, AST) were obtained. One step troponin I test was used for qualitative assessment of cTnI. Relevant clinical and demographic data were collected in a preformed proforma. Receiver Operating Curve was computed to assess diagnostic utility of various biomarkers, except for cTnI. Receiver Operating Curve could not be computed for cTnI as it was not quantitatively assessed. Statistics for tests of diagnostic utility were calculated for cTnI by 2 *2 tables. **Results:** The mean age of study group was 70.06 (10.11) and that of control group was 67.88 (9.96). Both groups were comparable in terms of gender distribution and history of smoking, diabetes and hypertension. Out of 50 cases 24 had presented to hospital within 6 hours. In the patients who presented early, the diagnostic performance of IMA was best (AUC= 0.801) followed by CK-MB (AUC=0.788) and AST (AUC=0.546). The ROC derived optimal cut off for IMA was 0.485 ABSU. At this cut off point sensitivity of IMA was 87.5%, specificity was 64%, PPV was 53.85% and NPV was 91.43%. In the current study, sensitivity of cTnI was 68%, specificity was 100%, PPV was 100% and NPV was 75.56%. **Conclusion:** IMA is a sensitive test with good NPV but a relatively modest specificity and PPV. Hence a negative IMA test seems to more helpful in ruling out the diagnosis of ACS in patients presenting with chest pain. However, a positive IMA test may not be as useful in ruling in the diagnosis of ACS. A positive cTnI test however virtually confirms the diagnosis of ACS because of its high specificity and PPV. However negative cTnI may not be as useful as negative IMA test. Both these test complement each other in assessment of ACS.

A-007

Performance Evaluation of Representative Immunoassays from the Cardiac Panel on the Alinity i System from Abbott Laboratories

C. Rudolph, K. Krishnan, J. Lee. *Abbott Diagnostics Division, Abbott Park, IL*

Background: Cardiovascular disease (CVD) is a broad term for a range of diseases affecting the heart and blood vessels. Abbott's Cardiac Panel of assays and analyzers provide accurate, reliable results with rapid turnaround time, aiding clinicians in providing better patient care. The Alinity i system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 200 tests per hour. The Alinity i system has an increased reagent load capacity, holding up to 47 immunoassay reagents, onboard QC, clot and bubble detection ability, and a dedicated pre-treatment lane to provide consistent and reliable results.

Objective: To demonstrate the analytical performance of representative assays of the Cardiac Panel of the Alinity i system, which consists of assays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for the quantitative determination of analytes in human serum and/or plasma.

Methods: Key performance testing including precision, limit of quantitation (LoQ), linearity, and method comparison were evaluated per Clinical and Laboratory Standards Institute (CLSI) guidelines. The inputs to the Analytical Measuring Interval include the observed LoQ, imprecision across the range, and the linear range.

Results: The observed results for LoQ, precision, linearity, method comparison to ARCHITECT using Passing-Bablok regression, and determined Analytical Measuring Interval for representative assays in the Cardiac Panel are shown in the table below.

Assay	LOQ	Total %CV[a]	Linear Range	Method Comparison (Slope/ Correlation)	Analytical Measuring Interval
STAT CKMB	1.0 ng/mL	2.9	0.0 to 321.2 ng/mL	0.94/1.00	1.0 to 300.0 ng/mL
STAT Myoglobin	3.5 ng/mL	5.4	0.0 to 1309.2 ng/mL	1.00/1.00	3.5 to 1200.0 ng/mL
Galectin-3	1.3 ng/mL	3.4	1.2 to 130.4 ng/mL	1.01/1.00	4.0 to 114.0 ng/mL
BNP	5.4 pg/mL	4.5	2.1 to 5271.1 pg/mL	0.95/1.00	10 to 5000 pg/mL
NT-proBNP	8.3 pg/mL	5.4	7.9 to 43414.2 pg/mL	0.96/1.00	8.3 to 35000.0 pg/mL

[a] Within-Laboratory (Total) variability contains within-run, between-run, and between-day variance components

Conclusion: Representative immunoassays from the Cardiac Panel utilizing CMIA technology on the Alinity i system demonstrated acceptable performance for sensitivity, precision, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT immunoassays.

A-008

Pentraxin 3 as a Marker of Inflammation and Atherosclerosis in Cholesterol-fed Rabbits

J. C. Gonzalez, L. M. Figueira, D. C. Gonzalez. *Carabobo's University, Valencia, Venezuela, Bolivarian Republic of*

Background: Inflammation plays an important role during the atherosclerosis. Different studies have been carried out on the relationship between inflammatory markers and the development of atherosclerosis, suggesting their utility to identify the risk of an acute ischemic event and the detection of vulnerable plaques. Pentraxin 3 (PTX3) and C- reactive protein (CRP) are acute phase proteins that belong to pentraxin family and play an important role in inflammatory reactions such as in atherosclerosis. CRP is the most recognized marker for cardiovascular disease. The evidence indicates that PTX3 exerts an important role in modulating the cardiovascular system in humans and experimental models. In fact, PTX3 is strongly expressed in atherosclerotic arteries and its high plasma levels were found to be related with severity of coronary atherosclerosis, thus, its represents a rapid biomarker for primary local activation of innate immunity and inflammation. However, has been described that PTX3 has a protective role in atherosclerosis, since it attenuates leukocytes recruitment at the site of inflammation. Therefore, the role of PTX3 in the atherosclerosis is has not been established so far. Thus, we were prompted to evaluate the role of plasmatic PTX-3 and CRP in determining the presence and severity of atherosclerosis. **Methods:** New Zealand white male rabbits were randomly divided into two groups, control group (CG) and experimental group (EG), receiving standard diet (commercial rabbit food) and water. The EG rabbits were fed a cholesterol diet (1% cholesterol) for 12 weeks. Blood samples of overnight-fasted rabbits were collected on basal, sixth and twelfth weeks, and plasma concentration of lipid profile, PTX3 and high-sensitivity CRP (hsCRP) were determined. Half of the animals were sacrificed by cervical dislocation on sixth or on twelfth week, and the aortic arch and descending aorta was dissected for histological studies. **Results:** Our findings demonstrated that cholesterol diet induced significant increases in lipid profile. The PTX-3 and CRP levels were significantly higher in EG than CG both on 6th and 12th week ($p < 0.001$). Moreover, cholesterol diet induced advanced atherosclerotic lesions (types III and IV) in the aortic arch and descending aorta. **Conclusion:** These results support that plasmatic PTX-3 and CRP levels were associated with the presence of atherosclerosis and it may be regarded as novel early markers of atherosclerosis.

A-009

A Highly Stable Cardiac Troponin I Recombinant Protein: An Ideal Candidate as cTnI Calibrator or Quality Control Material

L. Shen[1], Q. L. Yu[1], H. P. Huang[2], Q. H. Jin[1], C. H. Xiao[1], M. Yan[2], Z. J. Deng[2], Y. Y. Liu[2], L. Peng[2], J. Luo[2], B. Hu[2], R. B. Zheng[1], J. Ge[1]. *[1]Hunan Yonghe-sun Biotechnology Co., Ltd, Changsha, China, [2]Hunan Testing Insitute For Medical Devices, Changsha, China*

Background: Cardiac troponin I (cTnI), a polypeptide expressed in cardiac muscles with 110 amino-acid residues, is one of the subunits in the troponin complex (TnT, TnI and TnC) bound to tropomyosin and actin on the thin filaments of striated muscle fibres. Previous studies indicated that cTnI released from cardiac tissue may undergo molecular changes, and most of cTnI molecule may exist as cTnI-cTnC complex. This leads to the notion that antibodies in analytical systems may not equally recognize cTnI forms, resulting the difficulty of cTnI assay standardization. Through the cooperation of AACC and NIST, a natural troponin C-I-T complex (SRM 2921) provided by Hytest, which is demonstrated to improve harmonization and commutability of the different cTnI assay platforms, is suggested to be used as the cTnI reference materials for further study. However, the practicability of SRM 2921 is limited due to its poor stability. Therefore, we focused on preparation of a cTnI reference material which could be used for improving the stability of cTnI calibrator or QC material.

Methods: The cTnI recombinant protein (M.W. 30.22 kDa) was designed as a C-I complex (named as "ILCH"), having 273 amino acid residues, which consists of full fragments of cTnI, a linker, and partial fragments of cTnC. The ILCH gene was inserted into the expression vector pET-28a, and the plasmid was transformed into *E. coli* BL21(DE3). The ILCH protein was expressed under condition of 1 mM IPTG for 4 hrs at 30°C and purified by affinity chromatography. The comparative stability study of ILCH with 8T62 (the raw material of SRM 2921 obtained from Hytest, Finland) was performed under the condition of thermal accelerated experiment at 42°C. In the thermal acceleration study, four concentrations of ILCH and 8T62 were prepared: 1.0 μg/L, 5.0 μg/L, 25 μg/L and 50 μg/L, respectively. The samples were then placed into an electric thermostatic drying cabinet at 42°C up to 50 days. The treated ILCH and 8T62 were then tested and evaluated by FIA at set intervals until 50 days. The ILCH were also measured by using Beckman *Access2* cTnI assay.

Results: Our thermal acceleration results showed that the stability of ILCH was found much better compared with 8T62. For example, on Day 3, the degradation rate of 8T62 at 1.0 μg/L and 5.0 μg/L were 25.0%, and 25.9%, respectively. On Day 17, the degradation rate of 8T62 at 1.0 μg/L and 5.0 μg/L were 65.0%, and 61.6%, respectively. Notably, we found the degradation rate of ILCH at 1.0 μg/L and 5.0 μg/L were only 4.8% and -5.4% on day 17. Analytical values of ILCH were obtained from Beckman *Access2* cTnI assay, and the values were found very close to that obtained from FIA.

Conclusion: In this study, we describe a highly stable cTnI recombinant protein, ILCH, which could be an ideal candidate of cTnI calibrator or QC material for assay standardization of cTnI on different assay platforms.

A-010

Hs Troponin I Performance Characteristics on Alinity i in Karachi, Pakistan

F. Z. Kanani, A. H. Kazmi. *The Indus Hospital, Karachi, Pakistan*

Background: We recently shifted from conventional troponin I (c trop I) assay to high sensitive troponin I (hs trop I) assay on Alinity i by Abbott Diagnostics. Alinity i system has recently been launched internationally, and we are amongst the first ones in Pakistan to adopt the assay on this analyzer. We aimed to perform analytical evaluation of hs trop I on two Alinity i analyzers.

Method: Precision: This was verified by running commercial controls at three different levels in five replicates for five days.

Accuracy: External proficiency sample were run and results compared to Architect group mean.

Sensitivity: Limit of Blank, Limit of Detection and Limit of Quantification were determined and compared to vendor claims.

Method comparison: 40 samples were analyzed for c trop I on Vitros Eci and hs trop I on both Alinity i analyzers across entire analytical measuring range (AMR). Verification of 99th percentile URL: Hs trop I was performed on 40 healthy male voluntary blood donors (20 on the each Alinity i). HbA1c and estimated creatinine clearance of the donors were also determined to further define normality.

Results: Precision study showed a coefficient of variation (CV) of 5.6/ 5.7, 1.4/ 2.7 and 1.1/4.1 for Alinity i 1 and 2 at low, medium and high levels. The samples were

within total allowable error in the CAP proficiency testing survey when compared to Architect series, though with a negative bias. Limit of blank was 0.99 and 0.19 ng/L and limit of detection was 1.59 and 0.64 ng/L on Alinity i 1 and 2 respectively. Limit of quantification was verified to be less than 5.1 ng/L as claimed (4.4 and 3.6 ng/L). The assay demonstrated a CV of less than 6% at values corresponding to 99th percentile URL on both Alinity i. Passing Bablock between c trop I on Vitros Eci and hs trop i on the two Alinity i instruments showed a slope of 0.988 (r=0.99)and 0.921 (r=0.96), (95% CI included 1.0) with a negative intercept of 3.98 and 3.35, and no significant difference in mean concentrations. Hs trop I levels were below the gender specific cut offs in all healthy donors, being undetectable in 35 out of 40 males tested. It needs to be seen if 99th percentile URL levels are lower in our ethnic group.

Conclusion: The analytical evaluation of hs troponin Iiassay on two Alinity i analyzers was successful.

A-011

Robustness of the Troponin 0/1-h Algorithm for Early Diagnosis of Acute Myocardial Infarction when Measured on Two Different Instruments of the Same Type

A. Haushofer[1], J. Seier[1], M. Stübler[2], G. Lirk[3], A. Ziegler[4], E. Giannitsis[5], B. Lindahl[6], C. Mueller[7]. *[1]Central Laboratory, Klinikum Wels-Grieskirchen, Wels, Austria, [2]Central Laboratory for Molecular Diagnostics, Medical University of Graz, Graz, Austria, [3]University of Applied Sciences Upper Austria, Hagenberg im Mühlkreis, Austria, [4]Roche Diagnostics International Ltd, Rotkreuz, Switzerland, [5]Department of Internal Medicine III, Cardiology, Angiology & Pulmonology, Heidelberg University Hospital, Heidelberg, Germany, [6]Department of Medical Sciences and Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden, [7]Department of Cardiology and Cardiovascular Research Institute Basel, University Hospital Basel, Basel, Switzerland*

Background: Accelerated protocols for acute myocardial infarction (AMI) diagnosis, based on relatively low troponin concentrations and small acute changes of 3-5 ng/L over 1 h in blood, have been validated in several research studies. However, blood samples were typically measured on a single instrument. Therefore, evidence and guidance is required for routine operation and workflow in a centralized laboratory, where serial troponin samples from the same patient might be measured on two different instruments of the same type.

Methods: In this sub-analysis of the multicenter TRAPID-AMI study, we evaluated the performance of the accelerated 0/1-h AMI algorithm when measuring serial troponin samples from the same patient, with symptoms suggestive of AMI, on two parallel instruments. Patients eligible for inclusion provided informed consent for remeasurements and 707 samples were available. The 0-h and 1-h samples were measured with the Elecsys® Troponin T-high sensitive (cTnT-hs) assay on two different **cobas** 8000 analyzers (Roche Diagnostics). AMI diagnosis was determined by 0/1-h criteria for rule-out (cTnT-hs <12 ng/L and change <3 ng/L at 1 h) and 0/1-h criteria for rule-in (cTnT-hs >52 ng/L or change >5 ng/L at 1 h); remaining individuals were classified to the observation zone. Outcomes were analyzed for different data combinations, e.g. all samples measured or 0-h and 1-h samples randomly assigned, on the two different instruments.

Results: When running all samples from the same patient on two different instruments of the same type, results were in agreement for 691/707 (97.7%) samples analyzed: 354 rule-out, 88 rule-in and 249 observation zone. Results differed (instrument 1/ instrument 2) for 16 (2.3%) samples: 1 observation/rule-out; 1 rule-in/observation and 14 rule-out/observation. No reclassifications from rule-in to rule-out were observed and only 1 reclassification from rule-in to observation (0.1%). The potential variation introduced by the parallel instrument setup is thus small compared with variations introduced by an instrument switch when using traditional diagnostic protocols, e.g. a single cutoff.

Conclusions: The 0/1-h algorithm appears to be safe and effective for triaging patients with suspected AMI when measured on two different instruments of the same type.

Disclaimer: The claims discussed in this abstract have not been cleared or approved by the FDA.

The aforementioned authors have developed this abstract on behalf of the TRAPID-AMI investigators.

A-012

Measurement of Novel Adipokine Visfatin in Young Patients with Acute Myocardial Infarction

D. Stejskal, R. Šigutová. *University Hospital Ostrava nad Faculty of medicine Ostrava University, Ostrava-Poruba, Czech Republic*

Background: Fat tissue produces a variety of secreted proteins (adipocytokines) with important roles in metabolism. Recently was isolated a newly identified adipocytokine, visfatin, that is highly enriched in the visceral fat of both humans and mice and whose expression level in plasma increases during the development of obesity. Visfatin has been also proven to be a proinflammatory mediator involved in the process of atherosclerosis. Visfatin has been shown to play a role in plaque destabilization as it is found abundantly in foam cell macrophages within unstable atherosclerotic plaques. The aim was to evaluate an assay for the determination of Visfatin in human serum, and to investigate its clinical relevance as a marker of acute myoradial infarction in young population (Men under 45y, Women under55y).

Methods: We clinically tested a sandwich ELISA assay in young individuals with acute myocardial infarction (n=50). Control population were the healthy probands without inflammation, hepatic or renal injuries, under 55 years of age

Results: Visfatin in sera can differentiate healthy subjects from young patients with acute myocardial infarction (5 vs. 27 ng/L). Visafatin in sera of AMI probands, correlated with glucose, creatinine, hsCRP and uric acid. Healthy probands had no such correlations. ROC analysis: visfatin cut off concentration was 20 ng/l with sensitivity 86% and specificity 91%. AUC of cTNI was 0.96, AUC of visfatin was 0.96, dif not differ.

Conclusion: We conclude that visfatin in serum is new independent potencial marker of AMI

A-013

Data Mining: Biological Factors Associated with Blood Cardiac Troponin I Concentration in China

D. Li[1], D. Wang[2], D. Wang[1], C. Ma[1], J. Wu[1], P. Li[1], X. Guo[1], L. Qiu[1]. *[1]Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical Colle, Beijing, China, [2]Department of Obstetrics and Gynecology Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Science, Beijing, China*

Background: Cardiac troponin is the cornerstone biomarker for the diagnosis of acute myocardial infarction. The aims of this study were to evaluate the association of biological and temporal factors with plasma cardiac troponin I (cTnI) concentration in a large group of Chinese outpatients and to explore which of four factors (gender, age, time of blood sampling, and season of the year) has the biggest influence on plasma cTnI levels.

Methods: Analytical data with outpatient cTnI results were downloaded from the laboratory information system from January 1, 2012 to September 20, 2018. All cTnI levels were measured using a Siemens Dimension EXL automatic chemiluminescence immunoassay analyzer. A statistical method was used to strictly exclude outliers. A total of 86,381 outpatients were enrolled in the study.

Results: In subjects over 60 years old, cTnI levels gradually increased with age in both males and females. cTnI levels reached their highest values in subjects aged ≥ 80 years (0.030 µg/L in males and 0.027 µg/L in female). In subjects over 70 years of age, cTnI levels were significantly higher in males than in females ($P < 0.05$). cTnI concentrations varied in subjects with different times of blood sampling. Both in men and women, cTnI concentrations reached a maximum at 05:00 hours (0.030 µg/L and 0.026 µg/L, respectively) and peaked again at 20:00 hours (0.029 µg/L and 0.023 µg/L, respectively). Additionally, there were significant differences among the four seasons of the year (P < 0.05). In winter, cTnI levels were usually higher than those in spring. Linear regression analysis showed that the factor, age ≥g80, had the greatest impact on cTnI levels.

Conclusion: Plasma cTnI levels were significantly influenced by gender, age, time of blood sampling, and season of the year. Thus, in order to avoid incorrect identification of cTnI values as abnormal, a cTnI reference interval needs to be established, taking into consideration the gender and age of the subject, the time of day of blood sampling, and the season of the year.

A-015

Are We Meeting the 1 Hour Turnaround Time for Cardiac Troponin Testing as Recommended by the 2018 Expert Panel?

C. S. Lau, S. P. Hoo, S. F. Yew, S. K. Phua, T. C. Aw. *CHANGI GENERAL HOSPITAL, Singapore, Singapore*

Background: The 2018 American Association for Clinical Chemistry and International Federation of Clinical Chemistry (IFCC) expert panel recommended that cardiac troponin (cTn) results should be reported within 60minutes from sample reception. We describe our experience with turn-around-times (TAT) for high-sensitivity (hs)-cTnT results.

Methods: We are a 1000-bed acute care general hospital with 500-600 daily attendances at the emergency department (ED). Hs-cTnT is requested through the computerized physician order entry (CPOE) system. Upon blood collection (heparin) a barcode is generated (time 1), and samples are sent to the laboratory via pneumatic tubes. In the laboratory sample details are verified (time 2) and samples placed into the Cobas8000 (Roche) lab automation system (LAS). After centrifugation and decapping, plasma is aspirated for hs-cTnT testing (9 min assay) by electro-chemiluminescence immunoassay (Cobas e801). Results are auto-validated and transmitted to the electronic medical records (time 3). TAT from lab reception to reporting (R-R TAT = time3-time2) and collection to lab reception (C-L TAT = time2-time1) of samples from September 2018-January 2019 were analysed. MedCalcSoftwareV18.11 (Ostend, Belgium) was used for statistical analyses.

Results: Data from 31363 samples were reviewed; 29425 samples (93.8%) had R-R TAT ≤60min (table 1), with a median (IQR) of 34min (13-303). Out of 1938 cases whose R-R TAT >60min, 1212 (62.5%) cases occurred outside office hours. C-L TAT was ≤60min in 29560 (94.1%) of samples with a median (IQR) of 12min (1-1243). ED samples accounted for 16733 cases (53.4%) with a median (IQR) R-R TAT of 33min (14-163) and median (IQR) C-L TAT of 9min (1-369).

Conclusion: The laboratory is close to achieving the AACC-IFCC TAT goals for troponin testing, but gaps remain. Closer attention to after-office hour service and prompt delivery of samples to the laboratory is needed. Critical success factors include CPOE, heparin tubes, pneumatic tubes, LAS, and autovalidation.

Table 1: Distribution of TAT from Receipt-Results

R-R TAT (min)	N *(%)*	Cumulative N *(%)*
11 - 15	17 *(0.1)*	17 *(0.1)*
16 - 20	310 *(1.0)*	327 *(1.1)*
21 – 25	1382 *(4.4)*	1709 *(5.5)*
26 - 30	7425 *(23.7)*	9134 *(29.2)*
31 - 35	8672 *(27.7)*	17806 *(56.9)*
36 - 40	5182 *(16.5)*	22988 *(73.4)*
41 - 45	2652 *(8.5)*	25640 *(81.9)*
46 - 50	1791 *(5.7)*	27431 *(87.6)*
51 - 55	1168 *(3.7)*	28599 *(91.3)*
56 - 60	826 *(2.6)*	29425 *(93.9)*
>60	1938 *(6.2)*	31363 *(100)*

A-016

Relationship between Estimated Glomerular Filtration Rate and Serum Biomarkers of Cardiovascular Disease

L. Pang, H. Li. *Peking University First Hospital, beijing, China*

Background: Chronic kidney disease (CKD) is associated with an increased cardiovascular disease (CVD) mortality risk. The purpose of this study was to investigate the relationship between alterations in estimated glomerular filtration rate (eGFR) and serum biomarkers of CVD.

Methods: We examined the cross-sectional associations of eGFR and high sensitive cardiac troponin I (hs-cTnI), creatine kinase (CK), CK-MB, lactate dehydrogenase (LDH) and brain natriuretic peptide (BNP) in 812 individuals without overt CVD.

Results: There were significant differences of hs-cTnI, CK, CK-MB, LDH and BNP among eGFR < 60, 60 - 90 and ≥ 90 ml/min/1.73 m^2. There was a strong and significant negative correlation between eGFR and hs-cTnI, CK-MB, LDH, BNP whereas there was no significant correlation between eGFR and CK when eGFR was taken into consideration as a continuous variable. eGFR was associated with these biomarkers of CVD. For example, eGFR < 60 ml/min/1.73 m^2 (vs ≥ 90 ml/min/1.73 m^2) was significantly associated with a [ratio (95% CI, *P* value)] 11.22 (5.58-22.54, *P* < 0.001), 3.05 (1.83-5.09, *P* < 0.001), and 7.84 (4.93-12.45, *P* < 0.001) times higher hs-cTnI, LDH and BNP, respectively. After adjustment for potential confounders, eGFR was associated with a 2.83 (1.08-7.41, *P* = 0.035) times higher of elevated hs-cTnI.

Conclusion: Reduced eGFR is associated with elevated hs-cTnI, LDH and BNP among individuals without clinically evident CVD.

A-017

Performance Evaluation of a New Troponin T-high Sensitive Assay with Increased Tolerance to Biotin

R. Imdahl[1], G. Albert[2], S. Kunzelmann[3], C. Rank[3], R. Zerback[4], A. von Meyer[5]. *[1]Labor Augsburg MVZ, Augsburg, Germany, [2]Roche Diagnostics International Ltd, Rotkreuz, Switzerland, [3]Roche Diagnostics GmbH, Penzberg, Germany, [4]Roche Diagnostics GmbH, Mannheim, Germany, [5]Institute for Laboratory Medicine and Microbiology, Kliniken Nordoberpfalz AG, Weiden, Germany*

Background: Pending the results of ongoing clinical trials, future multiple sclerosis therapies might comprise high-dose biotin regimens (≤300 mg daily) that can produce high blood biotin concentrations, but no higher than 1200 µg/L. Biotin concentrations >20 µg/L may interfere with the existing Elecsys® Troponin T-high sensitive assay (cTnT-hs; Roche Diagnostics), which is used to measure cardiac troponin T (cTnT) to aid the diagnosis of acute myocardial infarction. In this study, we evaluated the performance of a new assay, referred to here as cTnT-hs*, which was designed to reduce biotin interference.

Methods: Assessments were performed using up to two assay applications (18 min and 9 min [STAT]) on three analyzers (**cobas e** 411, **cobas e** 601 and **cobas e** 801). Biotin interference with cTnT-hs* was determined by measuring recovery in a series dilution of 11 samples with biotin concentrations ranging from 0-3600 µg/L. Repeatability was evaluated in five serum sample pools (n=75 each). Method comparisons of cTnT-hs* vs cTnT-hs, cTnT-hs* 18 min vs 9 min, and cTnT-hs* on different analyzers were evaluated using Passing-Bablok regression and Pearson's correlation. Clinical concordance of cTnT-hs* vs cTnT-hs (n=300 lithium-heparin plasma samples) was calculated. Repeatability, method comparisons and clinical concordance were tested using samples that did not contain biotin.

Results: Recovery using cTnT-hs* (**cobas e** 601; 18 min) was ≥99% for biotin concentrations ≤500 µg/L, ≥96% for biotin concentrations ≤1250 µg/L, ≥83% for biotin concentrations ≤2000 µg/L, and ≥12% for biotin concentrations ≤3600 µg/L. For cTnT-hs*, coefficients of variation for repeatability in serum samples (mean cTnT concentrations ranged 8.528-9768 ng/L) were: **cobas e** 411, 1.0-13.8% (18 min) and 1.1-11.7% (9 min); **cobas e** 601, 1.1-2.6% (18 min) and 0.9-2.5% (9 min); **cobas e** 801, 1.2-4.9% (18 min) and 1.3-3.4% (9 min). High correlation was demonstrated for all method comparisons: cTnT-hs* vs cTnT-hs using the 18 min application on **cobas e** 601 (y=1.003x + 0.650; *r*=1.000) and **cobas e** 801 (y=0.999x + 1.04; *r*=1.000); cTnT-hs* using 18 min vs 9 min applications (**cobas e** 601; y=0.975x + 1.22; *r*=1.000); and cTnT-hs* on each of the three analyzers using 18 min or 9 min applications. Clinical concordance for cTnT-hs* vs cTnT-hs (**cobas e** 601; 9 min) was high using the global (excluding US) 14 ng/L cutoff, with 95.3% negative and 100% positive agreement; Passing-Bablok regression, y=0.973x + 0.959 (*r*=1.000).

Conclusion: The new cTnT-hs* assay provides substantially greater tolerance to biotin interference, without affecting the performance of the existing cTnT-hs assay, across all platforms evaluated. cTnT-hs* could be used for patients with multiple sclerosis taking biotin up to 300 mg daily without any special precautions.

A-018

Examination of the Analytical Performance of the New High Sensitive POC Assay PATHFAST hs-cTnI

E. Spanuth[1], P. Engesser[2], E. Giannitsis[3]. *[1]DIAneering Diagnostic Engineering & Research, Heidelberg, Germany, [2]Mitsubishi Chemical Europe, Düsseldorf, Germany, [3]Department of Medicine III, Division of Cardiology, University Hospital Heidelberg, Heidelberg, Germany*

Background: The main analytical criteria for high-sensitivity cardiac troponin (cTn) assays are (i) imprecision (CV) at the 99th percentile value < 10%, (ii) detectable values above the limit of detection (LoD) in > 50% of healthy individuals, (iii) higher

99th percentile cutoffs in males than in females whereas 99th percentile cutoffs in females were significantly lower than the overall cutoff. We aimed to evaluate high sensitivity criteria for the point of care cardiac troponin I (cTnI) assays using the PATHFAST™ platform (LSI Medience Corporation, Tokyo).

Methods: Plasma samples were measured using the PATHFAST hs-cTnI. An imprecision profile was obtained by serial measurement of pooled heparin plasma samples according to Clinical & Laboratory Standards Institute (CLSI) guidelines. The limit of detection (LoD) was evaluated according to CLSI EP17 A2. The proportion of detectable values above the LoD was established by using 474 plasma samples of healthy individuals (236 women and 238 men, mean age 51 ± 14 years, range 18 - 86 years), in whom chronic diseases or cardiac disorders were excluded. The 99th percentile values were determined for all individuals, as well as women and men separately.

Results: Imprecision (CV) at the 99th percentile value were < 6%. The imprecision profile revealed the following values (mean (ng/L/CV): 1.99/21.6%; 3.13/8.35%; 8.31/6.85%; 14.67/5.13%; 19.6/5.71%; Mean cTnI values were higher in males than in females: 6.11 (24.05) ng/L in males and 4.60 (42.84) ng/L in females. LoD was established according to CLSI EP17-A2 with 2.9 ng/L. In the healthy population (N=474) detectable values (% > LoD) were overall 366 (77.2%); women 165 (69.3%); men 201 (85.2%), respectively. According to CLSI C28-A3 the following 99th percentiles were obtained: overall 27.47 ng/L, males 31.3 ng/L, females 24.9 ng/L. Only slightly higher cTnI values were found in subjects aged >65 years compared to subjects aged <65 years. The highest values in males aged >65 years were 27.40 ng/L and did not exceed the manufacturer recommended 99th percentile cutoffs of 29 ng/L).

Conclusion: In this analysis, the PATHFAST hs-cTnI POC assay fulfilled the analytical criteria for high-sensitivity cTn assays with high analytical accuracy according to the guidelines and definitions of the AACC / IFCC. Analysis of gender-specific 99th percentile values demonstrated significantly higher cutoff levels in males than in females. The imprecision slightly above the LoD at 3.13 ng/L was found to be 8.35%. Detectable values above the LoD were different between males and females (85.5 and 69.3%).

A-019

Influence of Pre-Analytical Factors on Lipoprotein Measurement by NMR

K. A. Roberts, B. C. Leindecker, R. J. Scott, L. J. Donato, A. S. Jaffe, J. W. Meeusen. *Mayo Clinic, Rochester, MN*

Background: Low-density (LDL-P) and high-density (HDL-P) lipoprotein particle numbers are associated with risk of cardiovascular disease. The most common clinical method for measuring LDL-P and HDL-P is nuclear magnetic resonance (NMR) spectroscopy, which measures the emitted frequency of methyl-protons in a static magnetic field following a radio frequency pulse. Heretofore, impact of pre-analytical factors had not been thoroughly described.

Objective: To assess the influence of fasting status, use of gel separator serum collection tubes, and storage conditions on lipoprotein results generated by NMR.

Method: LDL-P, HDL-P, and low-density lipoprotein cholesterol (LDL-C) were quantified using a Bruker Ascend 600 NMR (Bruker, Billerica, MA) with AXINON software (numares AG, Regensburg, Germany). Stored stability was determined by measuring sera from 10 subjects immediately following collection and at specified intervals after various storage conditions. Fasting or post-prandial effects on results were investigated by drawing serum from 10 subjects after an 8-hour fast and again 3 hours after a meal. NMR spectra were gathered from 50 residual sera collected using gel-separator tubes and compared to values collected conventionally.

Results: All samples repeated within 20% after storage: ambient 8 hours, refrigerate 7 days and frozen 14 days. Mean ±SD fasting versus non-fasting concentrations of LDL-P were 1,488±379 compared to 1,452±317 nmol/L, HDL-P 37.2±6.8 compared to 36.2±7.4 mcmol/L and LDL-C 123±33 compared to 110±27 mg/dL (all not significant). However, NMR results from two non-fasting samples could not be quantified due to an unusually large methyl-proton signal (Figure 1, top). In a separate analysis, 96% of spectra obtained from gel separator tubes could not be quantified due to a small but predictable peak overlaying the methyl-proton signal (Figure 1, bottom).

Conclusion: While NMR spectroscopy is a powerful tool, interferences should be carefully considered. Samples from fasting subjects using non-gel tubes produce the most reliable results for measuring lipoproteins.

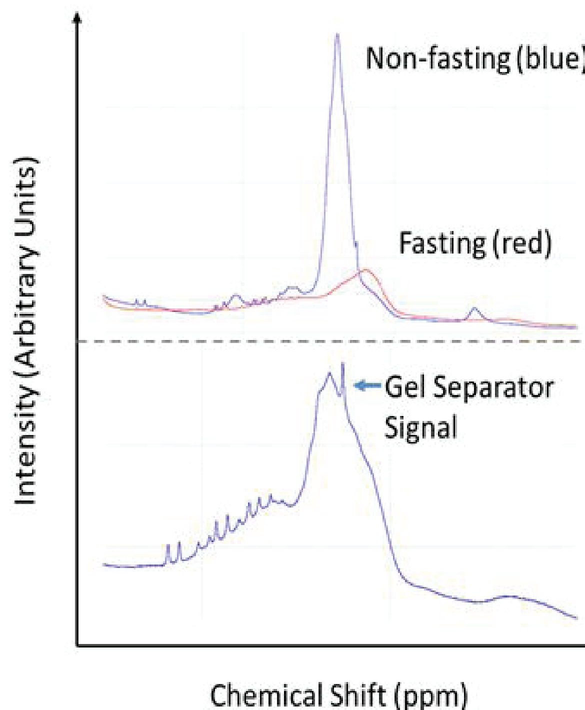


Figure 1: NMR Spectra

A-020

Implementing High - Sensitivity Cardiac Troponin T: The Issue of Hemolysis Interference

N. Y. Tang[1], Z. Mei[2], K. T. J. Yeo[1], X. M. R. van Wijk[1]. [1]*Section of Clinical Chemistry, Department of Pathology, Pritizker School of Medicine, The University of Chicagoty of Chicago, Chicago, IL,* [2]*Department of Pathology, Pritizker School of Medicine, The University of Chicago, Chicago, IL*

Background: Cardiac troponin T (cTnT) is one of the most sensitive and specific biomarkers of myocardial injury and acute myocardial infarction. However, hemolysis causes significant interference in the Roche Elecsys Troponin T Gen 5 STAT immunoassay. According to the Roche's package insert, a hemoglobin concentration > 0.1 g/dL (*i.e.* an H-index > 100 mg/dL) will lead to falsely depressed results. In our institution, 7.8% of specimens from the Emergency Department (ED) have an H-index > 100. Additionally, consecutive sample rejections were not uncommon. We thus performed hemolysis interference studies that would potentially allow us to increase the H-index limit and reduce sample rejection rate. Literature studies showed that enzymes, including cathepsin E, μ-calpain and thrombin could proteolyze cTnT. It has also been suggested that hemoglobin plays a role in mediating the negative interference. We aim to: (1) delineate a full hemolysis interferograph, (2) study the effect of protease inhibitors in hemolysate-affected samples and (3) determine the interference effect of hemoglobin alone.

Methods: Hemolysate was prepared using lysed heparinized O-negative blood. The hemolysate was mixed with a plasma pool with known cTnT concentration to create samples with H-indices ranging from 0-1600, followed by the measurement of cTnT in duplicates.To study the effect of protease inhibitors, a plasma pool with known cTnT concentration (~20 ng/L) was prepared and increasing amounts of hemolysate was added to create a hemolysate set. cTnT concentrations in hemolyzed plasma samples with the addition of protease inhibitors were compared to those in the absence of protease inhibitors. To determine to what extent hemoglobin *per se* contributes to the interference, "plasma + hemolysate" and "plasma + hemoglobin" specimens with hemoglobin concentrations of 0, 100, 300, 500, 700 and 900 mg/dL were prepared and the respective cTnT concentrations were compared.

Results: We found acceptable interference up to an H-index of 150, i.e. <10% depression of the cTnT result, which reduced our sample rejection rate from 7.8% to 4.1%. For samples with an H-index of 400, adding halt protease inhibitor 3 times more

than the manufacturer suggested amount (concentration 3X) could keep the recovery within +/- 10%. However, since this 3X concentration caused precipitation in plasma samples, it was unclear whether the precipitate has caused artifacts in the cTnT assay. Comparing the negative interference caused by hemolysate versus hemoglobin, hemoglobin alone contributed to at least 60% of the hemolysate interference when its concentration was ≥300 mg/dL. In addition, the extent of interference contributed by hemoglobin increased at higher hemoglobin concentrations.

Conclusion: Our interference studies allowed us to significantly reduce (47%) the ED sample rejection rate. Our result showed that hemolysis interference in the cTnT assay was mediated predominantly by the presence of hemoglobin, although proteolysis may also play a role. Ongoing studies will address whether hemoglobin removal could mitigate the hemolysis interference issue.

A-021

Validation of a NGS Pan Panel for Cardiomyopathies

F. S. V. Malta[1], R. L. M. Guedes[1], A. L. Júnior[2], D. Zauli[1]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute, Belo Horizonte, Brazil*

Background: Genetic markers have recently gained interest from physicians as they are dictating new peculiarities in cardiology, renovating tests applicability and the practical conducts. Advances in biotechnology have made available a variety of products and research possibilities on cardiomyopathies, resulting in a multitude of alternatives for doctors, patients and laboratories. Cardiomyopathies can be classified into five main groups with diverse clinical manifestations: I: Hypertrophic; II: Dilated; III: Left ventricle non-compaction; IV: Restrictive and V: Arrhythmogenic cardiomyopathy. They are characterized by complex molecular mechanisms, such as genetic heterogeneity, variable expressiveness, incomplete penetrance, multifactorial inheritance, digenic/oligogenic mechanisms, extensive genes and phenotype overlapping, which transform the genetic testing into a complex task, demanding multidisciplinary effort. **Objective:** Design and validate a Pan Panel for cardiomyopathies for clinical usage. **Methodology:** Ion AmpliSeq Designer was used to develop a Next Generation Sequencing (NGS) custom panel containing 47 genes focused in the distinct clinical presentations: I: Hypertrophic Cardiomyopathy (27 genes); II: Dilated Cardiomyopathy + left ventricular non-compaction cardiomyopathy (30 genes); III: Arrhythmogenic cardiomyopathy (12 genes); IV: left ventricular non-compaction cardiomyopathy (10 genes); V: Restrictive cardiomyopathy (8 genes) and VI: Global cardiomyopathy panel (47 genes, taking into consideration overlapping phenotypes). Control samples NA12878 and NA19240 of the Genetic Testing Reference Materials Coordination Program (GeT-RM) were obtained from Institute Coriell and sequenced in Ion S5™ platform and variant calling was performed by TVC plugin using Ion Server. Results were compared with variants retrieved from NCBI GeT-RM database. **Results and Discussion:** The panel presented an average of 95.5% of covered regions over 30X; however, the uncovered region represents approximately 11 kb due to the large panel size. The repeatability and reproducibility had low variability coefficients of 2.9% and 4.24%, respectively. The analytical sensitivity and specificity was 97.79% (CI: 95.26-99.19%) and 100% (CI: 100-100%), respectively. **Conclusions:** Uniformity inconsistency is a well-known NGS concern. The panel achieved a satisfactory performance and it can be applied into clinical practice, given that complementary sequencing should be considered for relevant uncovered regions. Clinically diagnosed patients with distinct cardiomyopathies will be sequenced for further evaluation.

A-022

Improved Low-End Precision of High-Sensitivity Troponin T (HS-TNT) on the Roche E801 Immunoassay System

C. S. Lau, S. P. Hoo, S. F. Yew, S. K. Phua, T. C. Aw. *CHANGI GENERAL HOSPITAL, Singapore, Singapore*

Background: The use of low values of high-sensitivity troponin T (hs-TnT) to rule out Acute Myocardial Infarction (AMI) in chest pain subjects is gaining traction. However, precision at these levels will be critical. We examined the precision of the Roche hs-TnT on the newly available immunoassay platform E801 (in use since August 2018) compared to the E601 (used from 2010-July 2018).

Methods: The Roche hs-TnT is an electro-chemiluminescence assay on the Cobas8000 total laboratory automation system. The quoted limit of detection (LoD) for hs-TnT on the CobasE601 is 5ng/L (package insert); the lowest mean hs-TnT concentration we verified (n=20) was 4.5±0.005ng/L with a coefficient of variation (CV) of 11.5% (95% confidence interval – CI: 8.7-16.8). We adopted 5ng/L as the hs-TnT LoD for E601. The lowest concentration reported on the Cobas E601 and E801 systems is 3ng/L. The imprecision of hs-TnT on the E801 was evaluated with pooled serum samples over a range of measured hs-TnT concentrations.

Results: The CV of hs-TnT on the E801 was much lower compared to the E601 (Table 1); at hs-TnT of 3.31ng/L a CV of 7.7% (95% CI: 7.2-8.2) was achieved. The LoD adopted for the E801 was 3ng/L. To corroborate the improved low-end sensitivity of the E801 hs-TnT we reviewed all hs-cTnT values of samples tested from August 2017 to January 2018 (Group A – E601) and from August 2018 to January 2019 (Group B – E801). The percentage of cases reported as below the LoD in Group A was 11.1% (4612/41501) whereas in Group B was 8.6% (3283/38145).

Conclusion: The precision and percentage of detectable hs-TnT on the E801 has improved over the E601. This will aid physicians in ruling out AMI in patients with chest pain. Besides, improved precision will be a boon in cardiac risk stratification when hs-TnT is used in community screening.

Table 1: Inter-Assay Precision (CV %) at different hs-cTnT concentrations.

Assay	N	Mean hs-cTnT (ng/L)	SD	CV (%)	CV (%) *[95%CI for CV]*
E601	20	4.5	0.001	11.5	11.5 *[8.7-16.8]*
	20	21.3	0.001	2.2	2.2 *[1.7-3.2]*
	20	60.9	0.002	2.7	2.7 *[2.0-3.9]*
	20	167.7	0.004	2.3	2.3 *[1.7-3.3]*
E801	20	3.3	0.254	7.7	7.7 *[7.2-8.2]*
	20	3.6	0.265	7.3	7.3 *[6.2-8.3]*
	20	6.8	0.249	4.0	4.0 *[3.4-4.5]*
	20	8.3	0.298	3.4	3.4 *[3.2-3.6]*
	20	9.8	0.266	2.9	2.9 *[2.3-3.4]*
	20	11.6	0.311	2.6	2.6 *[2.3-2.8]*
	20	15.6	0.281	1.9	1.9 *[1.3-2.6]*
	20	16.5	0.296	2.0	2.0 *[1.7-2.3]*
	20	21.1	0.417	1.5	1.5 *[0.8-2.2]*
	20	22.1	0.355	1.6	1.6 *[1.3-1.9]*

A-023

Validation of ESC 0/1-h and 0/3-h Algorithm for NSTEMI in Chinese Patients Attending Emergency Department

Y. Lin. *Beijing Key Laboratory for Molecular Diagnostics of Cardiovascular Diseases, State Key Laboratory of, Beijing, China*

Background: Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality worldwide. Use of high sensitivity cardiac troponin (hs-cTn) assay can improve the early diagnosis of AMI, especially non-ST-elevation myocardial infarction (NSTEMI). Current European Society of Cardiology (ESC) guidelines recommend 0-/1-hour and 0-/3-hour 'Rule-in' and 'rule-out' algorithms for NSTEMI by using hs-cTn. However, it lacks Chinese population data based on such diagnosis process. Thus, this study is to validate 1-hour and 3-hours diagnostic strategy using hs-cTnI (ARCHITECT) in Chinese patients with suspected NSTEMI.

Methods: From January to December in 2017, 580 patients with suspected ACS presenting to the emergency department were included. Patients aged 18-75 years without STEMI, major operation within 4 weeks, severe renal insufficiency (Ccr <30 ml/min), acute myocarditis or chronic heart failure. Serial measures of hs-TnI level were performed at 0 hour, 1 hour and 3 hours in patients with suspected AMI. The diagnosis of each enrolled patient will be made according to routine clinical approach and 1-hour and 3-hours clinical approach, respectively. The routine clinical diagnosis will be made by cardiologist panel according to third universal definition of myocardial infraction through reviewing all available medical records. The NSTEMI diagnosis depended on hs-cTnI(Architect) assessment will be made a senior cardiologist according to 1-hour and 3-hours clinical approach recommended by 2015 ESC guidelines for the management of NSTEMI. Finally, the positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity and a likelihood ratio(LR) are evaluated by using 0-/1-hour and 0-/3 hours algorithm. Statistical analyses were undertaken using MedCalc software version 15.2.2 (MedCalc Software, Mariakerke, Belgium).

Results: The age of the study population was 61.63±11.04 years; 156 patients (26.9%) were diagnosed with NSTEMI, and 197 patients(34.0%) and 227 patients(39.1%) were diagnosed non-ACS and unstable angina, respectively. According to ESC 0/1-h algorithm, 171 patients(40.1%) were diagnosed with NSTEMI.

The PPV, NPV and LR of 0/1-h algorithm were 85.4%, 99.6% and 0.01. The sensitivity and specificity was 99.3% and 91.0% for 1-hour algorithm. When ESC 0/3-h algorithm using a uniform and a sex-specific 99th centile diagnostic threshold were em-

ployed, 154(26.78%) and 151(26.26%) patients were classified as having NSTEMI. In uniform threshold approach, the PPV is 92.0%, the NPV is 97.4%, the sensitivity is 95.2%, and the specificity is 95.7%. Similarly, in sex-specific threshold approach, the PPV is 91.2%, the NPV is 96.0%, the sensitivity is 92.4%, and the specificity is 95.3%.

Conclusion: In emergency departments, all Chinese patients with chest pain of suspected ACS can be rapidly, safely and effectively classified into NSTEMI, non-ACS and unstable angina depending on ESC 0/1-h and 0/3-h Algorithm with hs-cTnI.

Keywords: High-Sensitivity Cardiac Troponin I, NSTEMI, Chinese Population

A-024

Utilisation of High Sensitivity Troponin in Routine Clinical Practice

P. O. Collinson, K. Hutt. *St George's Hospital, London, United Kingdom*

Objective: To assess routine ordering patterns for high sensitivity troponin requests in a University teaching hospital in a retrospective audit. The requesting protocol for troponins is based on the European Society of Cardiology (ESC) guidelines and incorporated into the Hospital Policy Guidelines document.

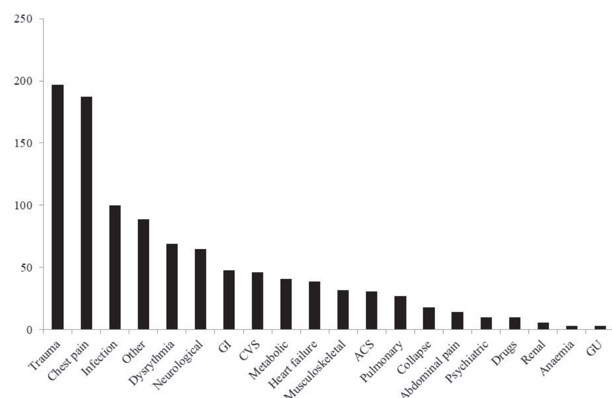
Methods: All troponin results coded from the Emergency Department (ED) for October 2018 where extracted from the laboratory information system. A list of patients attending plus attendance dates was then forwarded to the audit office and a corresponding list of matches plus clinically assigned diagnoses at presentation extracted from the ED information system. Diagnoses were then grouped into clinical categories based on organ system with cardiac cases subdivided according to likely clinical investigation categories.

Results: A total of 2839 requests were identified from 2394 patients, 1184 male (49.5%) median age 61.6 years (range 4.4-118.8) interquartile range (IQR) 42.1-78.1. The median number of troponin requests per patient was 1 (Range 1-5) IQR 1-1. 1656 had a single troponin ordered of which 1197 were below the 99th percentile (14 ng/L) and 449 below the LOD (3 ng/L)

Clinical diagnoses to match requests were available on 1219/2394 (50.9%) 572 males (46.9%) median age 64.6 years (range 13.9-118.7) IQR 45.0-79.4. The diagnosis subset had the same sex distribution but was significantly older (p = 0.02). Distribution of diagnosis is shown in the figure below.

Reviewing the request patterns for patients with chest pain (n = 187) 96 (51.3%) had a single troponin measured of which 45/96 (46.9%) were <3 ng/L allowing exclusion on admission. Of the remaining patients who had only a single troponin measured 42 had a troponin between 3 and 14 ng/L and 9 >= 14 ng/L.

Conclusion: There is widespread abuse of current requesting guidelines.



A-025

False Positive Elevation of Cardiac Troponin T Associated with Autoimmune Skeletal Muscle Injury

P. O. Collinson, P. Kiely. *St George's Hospital, London, United Kingdom*

Presentation: A female Asian age 65 years was referred for statin intolerance.

Past medical history: Type II diabetes mellitus (past 8 years) and had triple vessel coronary artery bypass grafting (2011) treated with atorvastatin 40 mg daily. When she attended the clinic she reported developing severe weakness whilst on holiday such that she could not get out of a car.

Medication at first consultation: Metformin 500mg bd, Zafirlukast 20mg od, Vitamin D3 (Cholecalciferol) 1000 units daily, Clopidogrel 75mg od, Omeprazole 20mg od, Losartan 50mg od, Ezetimibe 10 mg od, Tiotropium inhaler 18µg, Spiro base inhaler.

Investigations: All measurements were performed on a Roche Diagnostics Cobas system (Roche Diagnostics) except Cardiac Troponin I (cTnI, Architect, Abbott Diagnostics LOD 1.1 ng/L, 10% CV 4.7 ng/L,) . Noted to have elevated creatine kinase (CK) total 25-OH Vitamin D of 60 nmol/L. Cardiac Troponin T (cTnT) measured with a high sensitivity assay (LOD 3 ng/L, 10% CV13 ng/L) was elevated but no cardiac symptoms. Her serial results are shown in the table below.

Genetic testing (next-generation sequencing) excluded familial hypercholesterolemia with intermediate risk of polygenic hypercholesterolemia and the Rs4149056 genotype T/C (SLCOB1 gene, intermediate risk of myositis). A rheumatologist confirmed the diagnosis of myositis and performed an anti-muscle autoantibody screen, negative for typical anti-muscle antibodies but positive for an antibody to HMGCoA reductase. Her symptoms resolved with statin cessation with a fall in CK

The elevated cTnT with cTnI within the reference interval excluded myocardial injury. Polyethylene precipitation excluded a macrotroponin causing cTnT elevation. cTnT fell in parallel with CK. She was not rechallenged with a statin. Elevation of cTnT but not cTnI has been reported in conditions with muscle damage and muscle regeneration.

Conclusion: Auto immune myositis secondary to statin therapy with skeletal muscle regeneration and re-expression of a cross reacting TnT isoform.

Serial results						
	CRP (<3 mg/|L)	Creatinine (60-120 µmol/L)	ALT (<40 U/L)	CK (<200 U/L)	Troponin T (<14 ng/L)	Troponin I (<30 ng/L)
06-Sep-2018	1.0	53	41	699	25	7
19-Mar-2018	1.5	49	44	1101		
16-Oct-2017		43	38	1120		
15-Sep-2017	0.8	47	74	3770	122	5
31-Aug-2017		40	61	3904		
11-Jul-2017	108	48				
09-Jul-2017	9.5	55	47			
02-Jun-2017	1.0	44	55	2853	55	

A-026

A Baseline Novel High Sensitivity Cardiac Troponin I Level below the Limit of Quantitation Rules Out Acute Myocardial Infarction in the Emergency Department

B. Cook, J. McCord, M. Moyer, G. Jacobsen, R. Nowak. *Henry Ford Hospital, Detroit, MI*

Background: Cardiac troponin (cTn) measurements are used for the diagnosis of acute myocardial infarction (AMI). High sensitivity cTn assays allow for very early rule out of AMI, leading to more rapid diagnostic decision making. The objective of our study was to determine the utility of a baseline high sensitivity cardiac troponin (hs-cTnI) value (Access hsTnI, Beckman Coulter; Brea, California) below the limit of quantitation (LOQ) to rule out AMI in patients presenting to the Emergency Department (ED) with any suspicious symptoms of a cardiac etiology.

Methods: This was an observational trial enrolling subjects presenting to the Henry Ford Hospital (HFH) ED (Detroit, Michigan) with symptoms suspicious for AMI.

Informed consent was obtained and an ED physician clinically evaluated subjects by history, physical examination, ECG, blood testing (including hospital cTnI), and chest x-ray as appropriate. Blood specimens were collected within one hour after a triage ECG was obtained (within 10 minutes of presentation). Frozen plasma specimens (<80 degree C) were thawed once and hs-cTnI was measured using the Beckman Coulter Access 2 system in the clinical laboratory at HFH. Cardiac troponin I was measured using the Access hsTnI assay per manufacturer's instructions. Analytic performance testing for hs-TnI in HFH patients established a total imprecision (%CV) of 4.5% (26 ng/L), 6.2% (833.8 ng/L), and 6.0% (6605.8 ng/L). The Beckman Coulter published LOD (equals LoQ at 20% CV) and LoQ at 10% CV are 2.0 and 4.1 ng/L, respectively. The diagnosis of AMI was adjudicated by two cardiologists using the Third Universal Definition and Roche Diagnostics (Indianapolis, IN) Elecsys Troponin T Generation 5 assay on a Roche cobas e601 system with all available clinical data 30 days after presentation.

Results: 575 subjects were enrolled in the study with 567 having all data required for data analyses. AMI was diagnosed in 46 (8.1%) patients. 104 (18.3%) individuals had presentation hs-cTnI results <2.0 ng/L and 236 (41.6%) had values <4.1 ng/L. None of the patients with baseline hs-cTnI <2.0 ng/L had an AMI, yielding a negative predictive value (NPV) of 100.0% (CI 96.5 to 100.0%) and a sensitivity of 100% (CI 92.3 to 100.0%). Additionally, a presentation hs-cTnI value of <4.1 ng/L yielded a NPV of 100.0% (CI 98.4 to 100.0%), a sensitivity of 100.0% (CI 92.3 to 100.0%), and a good prognosis (no AMIs or cardiac-related deaths at 30 days).

Conclusions: In this single center ED study, a baseline presenting novel hs-cTnI value of <2.0 or <4.1 ng/L effectively ruled out AMI in 18.3% and 41.6% of all patients presenting to the ED and having any symptoms suspicious for AMI. Importantly all patients, not only those with chest pain, and those having symptoms for any duration or those with end stage renal disease and on dialysis were included. Additional studies are needed to validate our single baseline value <LoQ for AMI rule out and to determine the delta 1 or 2 hour values that might provide further guidance for managing ED patients with possible AMI.

A-027

Urine Derived Renal Cells as Tools to Diagnose Salt Sensitivity

K. A. Sarpong, R. A. Felder, J. J. Gildea. *University of Virginia School of Medicine, charlottesville, VA*

Background: The dietary sodium recommendation by the Institute of Medicine is intended as a 'one size fits all' recommendation, but it is becoming clear that each individual is genetically programmed with a 'personal index of salt sensitivity'. 25% of Americans are affected with salt sensitivity of blood pressure independent of blood pressure that results in significant increases in cardiovascular morbidity and mortality. Although the most reliable method to measure salt sensitivity is blood pressure responses to changes in dietary salt, low compliance with salt restricted diets reduces the effectiveness of this approach. We are validating a cell-based assay that utilizes dopamine 1 receptor (D1R) recruitment to the plasma membrane of living renal proximal tubule cells excreted into the urine to differentially determine the salt index of individuals.

Method: Individuals were categorized into two phenotypes, salt-sensitive and salt-resistant, as defined by a greater than 7 mm change in blood pressure in response to controlled randomized sodium intake of low (10 mmol/day) to high (300 mmol/day) sodium (Na^+). Renal proximal tubule Cells (RPTCs) were semi-purified (>95% purity) from study participants' urine samples by removing casts, cuboidal epithelial cells, and other contaminants utilizing a multi-step sieving procedure. Intracellular sodium-induced recruitment of the D1R from cytosol to the plasma membrane was measured using a selective D1R antibody. The enhancement of fluorescent signal (D1R recruitment) upon salt challenge directly correlates with an individual's personal salt index, which is a better measurement than the arbitrary designation of salt sensitive, salt resistant, and inverse salt sensitive. We are using rigorous validation diagnostic assay tools (sensitivity, specificity, limit of detection, linearity, accuracy, imprecision) to accurately measure salt sensitivity in all study participants.

Results: Increasing intracellular Na^+ resulted in a reduced D1R recruitment to the plasma membrane in salt sensitive individuals as compared to salt resistant individuals. Furthermore, there was a linear relationship between each participant's personal blood pressure response to a change in their sodium diet (($y = -0.0107x + 0.68$ relative fluorescent units (RFU), $R^2 = 0.88$, N = 12, P value = 0.0001) and angiotensin II-stimulated intracellular Ca++ ($y = -0.0016x + 0.0336$, $R^2 = 0.7112$, P value = 0.001, N = 10) concentration over baseline. Studies are ongoing in additional volunteers who are participating in salt sensitivity studies http://saltstudy.com. We are also developing and have published additional novel diagnostic tests for salt sensitivity involving micro RNAs.

Conclusions: Isolating RPTCs from urine provides a personalized cell-based diagnostic test of salt sensitivity index that offers advantages over a 2-week controlled diet with respect to cost and patient compliance. Furthermore, the linear relationship between the change in Mean Arterial Pressure and response to Na^+ regulatory pathways suggests that an individual's RPTC response to intracellular Na^+ is personalized and predictive. Our validated cell-based D1R recruitment assay will be useful in the diagnosis of salt sensitivity in an apparently healthy normotensive adult population.

A-028

Novel Neprilysin-Derived BNP Fragments in the Plasma of Heart Failure Patients Suggest Possible Insights for a Guided ARNi Therapy

E. E. Feygina[1], A. B. Postnikov[1], N. N. Tamm[2], M. N. Bloshchitsyna[2], O. V. Antipova[2], A. G. Katrukha[1], A. G. Semenov[1]. [1]*HyTest Ltd., Turku, Finland,* [2]*Moscow State University, Moscow, Russian Federation*

Background: Entresto™ - angiotensin receptor neprilysin inhibitor (ARNi) - is a novel heart failure (HF) therapy. It comprises the inhibitor of neprilysin (NEP) - a protease that is responsible for the degradation of a variety of vasoactive substances. Among other substrates A-type and B-type natriuretic peptides (ANP and BNP) are of particular importance. The augmentation of active ANP and BNP levels is considered to be a possible mechanism of cardiac function improvement by ARNi. One might suggest that products of NEP-mediated natriuretic peptides proteolysis could reflect the impact of NEP on their metabolism and serve to discriminate patients who will benefit from ARNi therapy. We have previously shown *in vitro* and in a rat model that BNP cleavage by NEP at 17-18 aar results in ring structure opening and the formation of the neo17-epitope - which is a novel proteolytic epitope with C-terminal Arg-17 (BNP-neo17 form). However, the presence of BNP-neo17 in human circulation has not been shown before. The aim of this study was to explore BNP-neo17 in the plasma samples of HF patients and compare its level to total BNP and N-terminal pro-BNP (NT-proBNP) levels.

Methods: EDTA-plasma samples were obtained from 32 patients, who were hospitalized for acutely decompensated HF (NYHA functional class II-IV), and none of whom had received NEP-inhibition based therapy. The total BNP level was measured by an automated SES-BNP assay (ET Healthcare Pylon BNP assay), and the NT-proBNP was detected with an in-house immunoassay that utilized the anti-NT-proBNP mAbs 29D12 and NT34 (HyTest Ltd., epitopes 5-12 and 25-32 aar correspondingly) and the Roche Cobas e 411 analyzer. BNP-neo17 was measured by an in-house immunoassay that was based on polyclonal antibodies that were specific to the neo17-epitope and the anti-BNP mAb 50E1 (epitope 26-32 aar).

Results: The total BNP level in 32 of the EDTA-plasma samples was 28.5-3384.5 ng/L (median 322.2, IQR 161.3-737.0). NT-proBNP concentrations were in the range of 195.3-95470.0 ng/L (median 2582.0, IQR 847.7-5015.5) when measured by Cobas and 711.2-156402.4 ng/L (median 17313.1, IQR 6322.6-25598.0) when measured by the 29D12-NT34 immunoassay. BNP-neo17 was detected in 19 of the plasma samples (59.3%) and its concentration was rather low: 1.7-37.3 ng/L (median 7.7, IQR 6.2-14.0). Its level correlated with the levels of total BNP ($r=0.784$, $P < 0.0001$) and NT-proBNP measured by Cobas and by the 29D12-NT34 immunoassay ($r=0.712$, $P < 0.0001$ and $r=0.784$, $P < 0.0001$ correspondingly). There was no correlation of BNP-neo17/total BNP with total BNP ($r=-0.17523$, $P=0.3374$) or NT-proBNP levels, measured by either assay ($r=-0.1026$, $P=0.5762$ and $r=-0.0888$, $P=0.6287$ for the Cobas and 29D12-NT34 immunoassays correspondingly).

Conclusion: We showed that the NEP-derived BNP-neo17 is present in the circulation of HF patients. The percentage of BNP-neo17/total BNP varies among individuals independently of total BNP or NT-proBNP levels. Considering that BNP-neo17 is generated from the active form of BNP by NEP, we speculate that BNP-neo17 might serve as an independent biomarker that reflects the impact of NEP on active natriuretic peptides levels and thus could be used to guide ARNi or other NEP-inhibition based therapy.

A-029

Evaluation of Troponin I High Sensitive (HS) Assay Across Beckman DxI, Siemens Centaur, Abbott Architect and Fujirebio Lumipulse Platforms

S. Narla[1], L. Pritchard[1], S. Augustain[1], A. Dider[1], P. Frantz[2], S. Thabius[2], M. Florent[2]. *[1]Covance Central Laboratories, Indianapolis, IN, [2]Covance Central Laboratories, Geneva, Switzerland*

Background: The requirements from IFCC (International Federation of Clinical Chemistry) to qualify a cardiac Troponin assay to be qualified as a high sensitive assay are that the, Assay must have analytical imprecision of <10% at the 99th percentile, Assay must be able to measure cTn above the LOD in >50% of the patients and the 99th percentile should be established with 300 M and 300 F. In this study we evaluated the performance of two FDA approved Troponin HS assays (Siemens Centaur and Beckman DxI Assays) and two CE marked Troponin HS assays (Abbott Architect and Fujirebio Lumipulse Assays).

Methods: Lithium Heparin Plasma and Serum were collected from healthy subjects (30 Female and 22 Male). All the 52 samples for each sample type collected were tested across four different platforms on the (DxI, Centaur, Architect and Fujirebio) to determine the percent of samples that these platforms can detect troponin above the LOD. Five levels of LH plasma and serum samples were prepared covering the LLOQ and the 99th percentile range by spiking with high native troponin I plasma and serum samples respectively. These samples were tested in replicates of 4 per day over 10 days on three different platforms to evaluate the CV at or below the 99th percentile.

Results:

	Units: ng/L	Siemens Centaur	Beckman DxI	Abbott Architect	Fujirebio Lumipulse
Troponin I HS	Limit of Detection (LOD)	1.6	2.0	1.1	2.1
	Lower Limit of Quantification (LLOQ)	2.5	2.3	10.0	8.6
% of results >LOD or LLOQ	Serum	42%*	67%*	60%	90%
	LH Plasma	42%*	67%*	59%	87%
99th percentile as per the Manufacturer	Serum	F=39.59, M=58.05, O=46.47	F=13.6, M=19.8, O= 18.1	F=15.6, M=34.2, O=26.2	F=21.4, M=29.4, O= 26.9
	LH Plasma	F=36.99, M=57.27, O=47.34	F=14.9, M=19.8, O=17.9	F=15.6, M=34.2, O=26.2	F=27.8, M=32.8, O=29.6
CV at or below 99th percentile (n=40)	Serum	3% at 26.7	5% at 13.9	8% at 13.0	SNP
	LH Plasma	3% at 34.7	8% at 9.1	8% at 12.5	SNP
SNP: Study Not Performed, *the instrument gave results as less than for any result below LLOQ					

Conclusion: DxI, Architect and Lumipulse assays were able to detect troponin >50% of subjects tested, the results from Centaur were inconclusive as the instrument did not give results between LLOQ and LOD. All three assays tested DxI, Architect and the Centaur assays have %CV <10% at the 99th percentile concentration. Overall all assays evaluated meet the IFCC requirements to be qualified as a HS Troponin assay.

A-030

Full-Size Human Cardiac Troponin T and Its Fragments in the Plasma of Patients with Acute Myocardial Infarction

I. Katrukha[1], A. Vylegzhanina[1], A. Kogan[1], K. Mukharyamova[1], A. Bereznikova[1], A. Bogomolova[2], E. Koshkina[3], A. Katrukha[1]. *[1]HyTest LTD, Turku, Finland, Turku, Finland, [2]School of Biology, Moscow State University, Moscow, Russian Federation, [3]67-th City Hospital, Moscow, Russian Federation*

Background: The blood measurement of cardiac troponin T (cTnT) is one of the most reliable methods of acute myocardial infarction (AMI) diagnosis. cTnT is known to be susceptible to the proteolytic degradation, but there is still no consistent data describing the level and sites of degradation of cTnT molecules that are present in the blood of AMI patients. Meanwhile, degradation might have a significant influence on the precise immunodetection of cTnT. In this study we aimed to border the cTnT fragments present in the blood of AMI patients and to quantify their relative abundance at different time periods after AMI.

Methods: Serial heparin plasma samples were collected from AMI patients over a period of 3-31 hours following the onset of AMI. cTnT and its fragments were studied by means of Western blotting and sandwich immunofluorescent analysis (IFA) that utilized monoclonal antibodies (mAbs) which were specific to different portions of the cTnT molecule.

Results: In the blood of AMI patients we were able to discriminate approximately 23 proteolytic fragments of cTnT with relative molecular masses of 10-30 kDa, along with "full-size" 2-287 amino acid residues (aar) cTnT of ~37 kDa. Three major regions of cTnT degradation were observed: The first one was between aar 68 and 69, the second one - near aar 164 and the third one (which contained several sites of degradation) was between aar 190 and 223. Immunostaining with mAbs that were specific to the N-terminal and central parts of cTnT revealed the formation of fragments with relative molecular masses of 25-29 kDa (the most abundant fragments were ~2-164, ~2-190 aar, ~2-223 aar and 69-287 aar) and of 12-20 kDa (the most abundant fragments were ~69-164, ~69-190 to ~69-223 aar). Immunostaining of cTnT with mAbs that interact with the C-terminal part of cTnT (epitopes lay within 223-288 aar) showed the presence of seven C-terminal fragments of 8-14 kDa that appeared as a result of cleavage at several sites located between aar 190 and 223.

Immunostaining of cTnT from serial samples of AMI patients with the mAb 329 that was specific to the central part of the molecule (epitope 119-138 aar) revealed that the ratio of the full-size cTnT (2-287 aar) decreased by more than one half from ~65% at the early time point (5±1.7 hours after AMI, mean±SD) to ~25% at the late time point (29±3.2 hours after AMI). The ratio of the 25-29-kDa fragments remained unchanged and represented ~15% of all detected cTnT. The ratio of the 12-20 kDa central fragments increased from ~10% to ~55% (among those the ratio of the shortest fragment (aar 69-164) increased from ~5% to ~15%).

Conclusion: Taken together, the data suggest that the region located approximately between aar 69 and 164 of cTnT is a promising target for the antibodies to be used in cTnT immunoassays.

A-031

Magnetic Force-Assisted Electrochemical Sandwich Immunoassays of Cardiac Markers on the markB® Immunoassay System

H. Hwang, J. Kim, H. Kim, Y. Son, J. Choi. *BBB Inc., Seoul, Korea, Republic of*

Background: To examine analytical performance of markB® point-of-care immunoassay platform for the quantification of cardiac markers, such as cardiac troponin I (cTnI), creatine kinase-MB (CK-MB), and myoglobin, in human blood. Quantification of cardiac markers has been used to help diagnosis of acute coronary syndrome or acute myocardial infarction, and risk stratification of patients with chest pain. Herein, we develop and evaluate markB®, a portable electrochemical immunoassay platform, for the quantification of cTnI, CK-MB, and myoglobin in human blood. markB® is a fully integrated immunoassay system based on BBB's passive plasma separation technology and magnetic force-assisted electrochemical sandwich immunoassay (MESIA).

Methods: Once a drop of blood sample is loaded into a markB® strip, pure plasma is spontaneously separated and flows into a microchannel. The plasma dissolves prespotted paramagnetic nanoparticles, which are detection probes coated with gold and monoclonal target-specific antibodies. The channel contains carbon electrodes, on which monoclonal capture antibodies are immobilized. Target analytes in plasma bind to the antibodies coated on the detection probes, and at the same time form sandwich immuno-complexes on the electrode. External magnetic fields facilitates the reaction process, then removes unbound magnetic nanoparticles from the electrode. Finally, electrochemical redox signal from gold is measured by 3-electrode cyclic voltammetry to quantify the amount of target analytes forming the sandwich immuno-complexes on the sensor electrode. The analytical performances of markB® for assays of three cardiac markers - cTnI, CK-MB, and myoglobin, are evaluated by following the CLSI guidelines.

Results: One test result could be obtained within around 12 min. The measured data could also be transferred to a server to store, analyze, and share with others, as markB® has a mobile communication module and an independent power source. According to the preliminary evaluation of analytical performance of markB® for the cardic markers, Limit of quantification for cTnI, CK-MB, and myoglobin were determined to be 0.05, 1, and 5 ng/mL, respectively. Acceptable linearity ($r > 0.99$) and precision (< 10%CV) were achieved for each marker in the defined measuring intervals. No cross-reactivity was observed, nor was there interference to 20 substances, including

bilirubin, hemoglobin, triglyceride, total protein, rheumatoid factor, and HAMA, at the clinically high concentration.

Conclusion: markB® showed acceptable analytical performances to quantify cardiac markers including cTnI, CK-MB, and myoglobin in human blood samples. It would be useful for quantitative measurements of cTnI, CK-MB, and myoglobin in a small volume (~ 30 µL) of specimen at the point-of-care setting as well as in the clinical laboratories.

A-032

Analytical and Clinical Evaluations of the Elecsys 5th GEN Cardiac Troponin T Assay

M. A. Barbhuiya, E. C. Pederson, M. L. Straub, S. C. Scott, T. L. Neibauer, W. F. Salter, M. H. Creer, Y. Zhu. *Department of Pathology and Laboratory Medicine, Pennsylvania State University College of Medicine, Hershey, PA*

Background: With the FDA approval of the 5th GEN Cardiac Troponin T (cTnT) assay, many clinical laboratories are now implementing the new assay. However, how the 4th and 5th GEN assays compare and how it impacts on clinical decision making still remain to be adequately addressed. The objective of this study was to evaluate the analytical and clinical performance of the 5th GEN assay by comparing it with the 4th GEN assay.

Methods: Both cTnT assays were performed on the Roche e602 analyzer using heparinized plasma. Twenty each of control samples with target means of 16, 101, and 1760 ng/L were run for within-run and between-run precision analysis. The analytical measurement range (AMR) study was carried out using five samples (8, 16, 101, 2160 and 8600 ng/L) run in duplicates. For quantitative comparison, 44 samples were analyzed with the two assays. Qualitative comparison was carried out based on the 99th percentile thresholds of the 4th and 5th GEN assays for randomly selected patients with cTnT orders and patients with elevated N-terminal pro-brain natriuretic peptide (NT-proBNP), procalcitonin, and decreased estimated glomerular filtration rate (eGFR). The quantitative data were analyzed with EP Evaluator. Two-tailed Fisher's exact test was used for categorical data. The significance level was $P < 0.05$.

Results: The coefficient of variations of within-run and between-run were 0.6% - 2.3% and 3.1% - 4.0%, respectively. The linear regression analysis between measured and assigned values of five samples with known cTnT concentrations ranging 8 - 8600 ng/L showed excellent linear relationship: y (observed) = 0.980 x (assigned) + 2.291. Deming regression analysis of the results measured by the 4th and 5th GEN assays showed that the two methods compared well with each other: Y (5th) = 0.923x (4th) + 28.6, r = 0.9994, n=44). However, for samples with cTnT <150 ng/L, the 5th GEN assay had a constant positive bias with an average of 29 ng/L. The qualitative comparison demonstrated an agreement of 88.9% between the two methods. In this small pool of patient population (N=44), the cTnT concentrations of 3 myocardial infarction (MI) were all above the 99th percentile cutoff values of both assays. In patients with NT-proBNP >500 pg/ml, procalcitonin > 2.0 ng/mL, and eGFR < 30 mL/min/1.73 m^2, the percentages of elevated cTnT cases for 4th and 5th GEN assays were 56.7% vs 80% (P= 0.0946), 65% vs 65% (no difference) and 84.8% vs 91% (P= 0.7085) according to their 99th percentile cutoff values.

Conclusion: The 5th GEN assay demonstrates excellent precision and wide AMR compared to the 4th GEN. The 5th GEN assay has a better precision for samples with cTnT < 20 ng/L. Also, the 5th GEN assay values are constantly higher than the 4th GEN for samples with concentrations of cTnT <150 ng/L. Both assays identify comparable number of patients with elevated cTnT and same number of MI cases. Also, they detect similar portions of elevated cTnT in patients with increased NT-proBNP, procalcitonin, and decreased eGFR, but studies with a larger sample size are needed.

A-033

Analytical and Clinical Correlation of Troponin-I and D-Dimer between the POCT AQT90 FLEX and Central Automated Analyzers in a Hospital Setting

J. Buse, E. Carlson, J. Feser, K. Hoffarth, C. Sarty, S. Vu, B. Mali. *Saskatchewan Health Authority, Regina, SK, Canada*

Background: Diagnosis in an acute care setting requires the rapid and accurate measurement of biomarkers for appropriate triaging of patients. The American College of Cardiology and American Heart Association Guidelines state that either cardiac troponin I or T are the preferred biomarkers for diagnosing acute myocardial infarction (MI), while the American Society of Hematology guidelines state that using D-dimer as the initial test reduces the need for diagnostic imaging for patients at low risk for venous thromboembolism. Although provision of such testing on automated central analyzers in tertiary hospital centres is common, implementation in a rural, urgent care setting requires use of either near-patient or point of care testing.**Aim:** Validate troponin I and D-dimer assays on the AQT90 Flex and correlate patient results with both clinical diagnosis and central laboratory analyzers.

Methods: Left-over specimens from 248 patients were identified for this study over fifty, non-consecutive days. Plasma specimens from EDTA tubes were paired with EDTA whole blood specimens for measurement of troponin-I. Platelet poor plasma (PPP) in 3.2% buffered sodium citrate tubes were paired with EDTA whole blood specimens for measurement of D-dimer. Specimens used for this study were identified in the LIS from patients with troponin-I and/or D-dimer orders. For troponin-I, the initial order as well as subsequent orders up to 24 hours were analyzed on an Abbott Architect using EDTA plasma and AQT90 Flex cardiac troponin I assay using EDTA whole blood. For D-dimer, only the initial D-dimer order was analyzed on an automated coagulation analyzer [ACL TOP (IL)] D-dimer HS 500 using platelet poor plasma and AQT90 Flex D-dimer using EDTA whole blood. Method validation was carried out using quality control specimens for imprecision studies, and left-over patient specimens for linearity, carry-over and correlation studies.

Results: A total of 282 specimens were included in this study; 39 specimens for D-dimer analysis and 243 specimens for troponin-I analysis. The AQT90 Flex troponin I and Abbott Architect high-sensitivity TnI assay results showed 85% concordance with decision points of 23 ng/L and 30 ng/L; respectively. Discordant results were in the setting of MI, previous MI, heart failure, pneumonia, meningitis, pleural effusion, unstable angina, sepsis, respiratory failure and atrial fibrillation. Using the clinical diagnosis, the AQT90Flex troponin I test showed comparable sensitivity and specificity, 87% and 96% respectively, to the Abbott Architect high-sensitivity TnI Assay, 98% and 85% respectively. The AQT90 Flex D-dimer and ACL TOP (IL) D-dimer assay results showed 97% concordance with decision points of 650 and 500 ng/mL; respectively. The discordant result between analyzers occurred in the setting of vancomycin resistant enterococcus pneumonia, with no diagnosis of venous thromboembolism.

Conclusion: Good concordance between results, sensitivity and specificity from central laboratory analyzers and the AQT90 Flex troponin I and D-dimer assays was observed. The correlation of AQT90 Flex troponin I and D-dimer results to clinical presentation demonstrates their potential benefit to patient care in a rural, urgent care setting.

A-034

Misclassification Rates for Baseline Samples between High-Sensitivity Cardiac Troponin I and T Assays Using Sex-Specific Universal Sample Bank 99th Percentiles in Patients Presenting to the Emergency Department

K. M. Schulz[1], Y. Sandoval[2], S. Smith[1], A. Saenger[1], R. Gray[1], A. Jaffe[2], N. Mills[3], J. Nicholson[1], A. Sexter[1], F. Apple[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Mayo Clinic, Rochester, MN, [3]University of Edinburgh Centre for Cardiovascular Science, Edinburgh, United Kingdom*

Background: The goal of this study was to determine misclassification rates, based on sex-specific 99th percentile upper reference limits (URLs) derived from the AACC Universal Sample Bank (USB), between high-sensitivity cardiac troponin I (hs-cTnI) and Gen 5 (hs)-cTnT assays in a US emergency department undergoing cTnI measurements on clinical indication.

Methods: Plasma (EDTA) specimens (n=500) randomly selected from >3,000 baseline specimens from patients enrolled in the 'COmparisoN of High-sensitivity Cardiac TRoponin I and T ASsays' (CONTRAST) Trial (clinicaltrials.gov NCT03214029). Patient clinical information was not included. Fresh specimens were measured by the hs-cTnI Abbott Architect assay and the Gen5 cTnT Roche Cobas e601 assay (similar to the hs-cTnT used outside the US but limited in reporting at <6 ng/L). Sex-specific USB 99th percentile URLs were: Abbott - men 19 ng/L, women 10 ng/L, 18 overall ng/L; Roche - men 16 ng/L, women 10 ng/L, 16 overall ng/L. Proportions of increased results between assays were cross-tabulated to determine agreement with the Kappa statistic.

Results: The percentage of samples with results below each assay's LoD were: Abbott hs-cTnI (LoD 1.9 ng/L) 18.6%, Roche Gen 5 cTnT (LoD 6 ng/L) 21.4%. 105 specimens (21%) showed a discrepancy between the 2 assays using sex-specific URLs, compared to 100 (20%) using the overall URLs. The percentage of specimens above the sex-specific URLs was 33.6% for hs-cTnI compared to 49.4 for Gen 5 cTnT; a 15.8% difference between assays. With the sex-specific URLs, an additional 17.5% of women had increased values for the hs-cTnI vs. the Gen 5 cTnT. Based on sex-speciifc URLs a kappa of 0.58 (95%CI, 0.51, 0.65) was observed.

Conclusions: We demonstrate substantial differences between the Abbott hs-cTnI and Roche Gen 5 cTnT assays in the proportion of patient baseline values above the

LoD and the sex-specific USB 99th percentile URLs. There were a greater number of increases in the number of women detected using sex-specific URLs compared to the overall URL for each assay. Using sex-specific URLs will impact clinical decision making.

A-035

Single High-Sensitivity Cardiac Troponin I Measurement Used to Rule-Out Acute Myocardial Infarction at Presentation in Low Risk in Patients Evaluated in the Emergency Department

A. Saenger[1], K. M. Schulz[1], W. Peacock[2], Y. Sandoval[3], A. Sexter[1], J. Bishop[4], E. friedland[4], F. Apple[1]. *[1]Hennepin County Medical Center, Minneapolis, MN, [2]Baylor College of Medicine, Houston, TX, [3]Mayo Clinic, Rochester, MN, [4]Singulex, Inc., Alameda, CA*

Background: High sensitivity cardiac troponin (hs-cTn) assays are able to quantitate low concentrations of cTn and provide an opportunity to rule out acute myocardial infarction (MI) at an early stage following a patient's presentation to an emergency department. The objectives of the current study were to examine the performance of single hs-cTnI measurement strategy to rule out acute MI and predict 30-day safety outcomes at presentation in these patients.

Methods: This was a prospective, observational study of patients (n = 415) presenting to US emergency departments with suspected acute coronary syndrome in whom cTnI measurements were obtained using the Sgx Clarity cTnI (hs-cTnI) assay (Singulex). The Sgx Clarity cTnI assay is indicated to be used in conjunction with clinical evaluation for ruling out cardiac ischemia in patients suspected of having coronary artery disease. Clinical data and hs-cTnI results were analyzed to determine to allow: 1) clinical sensitivity and negative predictive value (NPV for ruling out acute MI) and 2) safety outcomes of acute MI and death at 30 days, using the highest hs-cTnI above the limit of detection (LoD; 0.08 ng/L) concentration that provided $\geq$99% sensitivity and $\geq$99.5% NPV.

Results: Acute MI occurred in 79 patients (19.0%) and 82 patients (19.8%) had an adverse outcome. The highest, optimal concentration to allow safe rule out was 2 ng/L, with 1 missed MI. In patients with a hs-cTnI <2 (n=199, 48%), the clinical sensitivity and NPV for acute MI were 98.7% (95% CI 96.3,100.0) and 99.5% (CI 98.5, 100.0), respectively. Further, the sensitivity and NPV for the safety outcome of acute MI or death within 30 days for hs-cTnI <1 ng/L were 98.8% (CI 96.4, 100.0) and 99.1% (CI 97.4, 100.0), respectively; with 1 missed event.

Conclusion: A strategy of using a single hs-cTnI <2 ng/L at presentation allowed the immediate identification of 48.0% of patients highly unlikely to have acute MI and who were at very low risk for events at 30 days.

A-036

Development of a Liquid-Stable High-Sensitivity Troponin Calibration Verification Control Kit to Characterize Method Linearity and Validate the Reportable Range

D. Avery, J. Pawlak, M. Sweatt, R. Ito. *LGC Maine Standards, Cumberland Foreside, ME*

Introduction: Troponin is a complex of C, I and T regulatory proteins and is an important cardiac biomarker. Following cardiac muscle damage, specific cardiac isoforms of troponin (TnI and TnT) are released into the blood stream and can be monitored to identify episodes of myocardial infarction (MI). With the introduction of high sensitivity troponin assays, low levels can now be detected within an hour of cardiac damage.

To support this low level testing, high sensitivity troponin assays have been developed by IVD manufacturers and were cleared by the FDA as non-waived laboratory tests. Therefore, in accordance with CLIA '88, clinical laboratories are required to verify the manufacturer's assay performance characteristics, including accuracy, precision and reportable range (AMR).

LGC's objective was to develop a five-level, liquid-stable, human serum based, high sensitivity troponin control kit to meet the calibration verification and linearity needs of clinical laboratories.

Methods: VALIDATE® hsTNT (405ro) was formulated in a human serum matrix according to CLSI EP06-A into five equal-delta concentrations to cover the reportable range of Roche's Troponin T Gen 5 assay: 0.006 - 10ng/mL.

For each level, samples were tested in triplicate on the Roche cobas® 6000 analyzer. Reported recoveries were evaluated for mean, SD, and linearity using MSDRx®, LGC's proprietary linearity software. Limits were applied as 50% of the total allowable error (TE_a) for the analyte: 1ng/L or 11%, whichever is greater. To establish product stability claims, samples were subjected to one freeze-thaw cycle, followed by a 24 hour, 22°C stress condition and four subsequent freeze-thaw cycles. A second VALIDATE® product was similarly formulated for hsTnI and was tested on Beckman Coulter's Unicel® DxI ACCESS hsTnI assay (0.002 - 27 ng/mL)

Results: For VALIDATE® hsTnT, all levels were stable, recovering within ±10% of the recovered values on the date of manufacture in both stress and real-time stability studies. Linear regression analysis of theoretical versus mean recovered values was $y= 0.996x + 0.006$, $r^2= 0.9999$. Typical recovered values for Level 1 and 5 were 0.01 to 9.56ng/mL. The recoveries were linear and within applied TE_a limits through the manufacturer's reportable range.

The VALIDATE® hsTnI formulation also demonstrated a linear response across the assay's reportable range; stability assessments are on-going.

Conclusion: VALIDATE® hsTnT, as a five level, liquid-stable, ready-to-use, control kit is fit-for-purpose as a calibration verification material for the Roche cobas® Troponin T Gen 5 (hsTnT) assay. The product is listed with the FDA and is commercially available.

A-037

Validation of *TREML4* mRNA Expression and Polymorphisms in Blood Leukocytes as Potential Biomarker of Atherosclerosis

V. H. R. Duarte[1], A. B. Manfredi[2], J. N. Araújo[1], J. B. Borges[2], G. M. Bastos[2], M. H. Hirata[3], R. D. C. Hirata[3], A. D. Luchessi[1], V. N. Silbiger[1]. *[1]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2]Instituto Dante Pazzanese de Cardiologia, São Paulo, Brazil, [3]Universidade de São Paulo, São Paulo, Brazil*

Background: Atherosclerosis is a chronic and progressive inflammatory disease that can remain asymptomatic throughout life until an acute cardiovascular event occurs. The detection of atherosclerosis in its subclinical phase is a priority for the primary prevention of coronary artery disease (CAD). In previous studies, our research group found that the mRNA expression of receptor Trem-like transcript 4 (TREML4) was significantly increased in blood leukocytes of acute coronary syndrome patients. Furthermore,TREML4 mRNA expression was influenced by gene polymorphisms and was associated with the severity of atherosclerotic lesions in coronary artery disease patients. Considering the good potential of TREML4 as a biomarker of the atherosclerotic burden, we aimed to validate TREML4 polymorphisms and mRNA expression in a group of individuals with subclinical atherosclerosis.

Methods: One hundred and thirty-two patients, aged 29 to 75 years old, with subclinical atherosclerosis identified by four methods: coronary artery calcium, carotid Doppler ultrasonography, ergometric test, ankle-brachial index. Peripheral blood samples were collected for biochemical tests, DNA and RNA extraction. Total RNA was extracted from blood leukocytes using RiboPure Blood kit. DNA synthesis was performed using the High Capacity cDNA Reverse Transcription Kit. TREML4 mRNA expression was analyzed by real-time PCR. The reference gene selected was ACTB. Genomic DNA was isolated using the QIAamp DNA Blood Mini Kit. TREML4 polymorphisms rs2803495 (A>G) and rs2803496 (C>T) were genotyped by real-time PCR using the TaqMan SNP Genotyping Assays and the Rotor-Gene Q Real-Time PCR Detection System. The prognostic and diagnostic score of suspected CAD was assessed using the Duke Treadmill Score.

Results: Subclinical atherosclerosis patients carrying rs2803495 G allele (AG+GG genotypes) are more likely to express TREML4 than AA genotype carriers (OR = 9.007, 95%CI = 4.1-19.8, p < 0.001). However, rs2803495 variant was not associated with the degree of TREML4 mRNA expression (p > 0.05). Analysis of the TREML4 rs2803496 variant showed that carriers of C allele (CT+CC genotypes) are more likely to expressTREML4 than subjects carrying TT genotype (OR = 11.1, 95%CI = 4.8-25.4, p < 0.011). Moreover, C allele was associated with high mRNA expression levels (O.R.=3.8, 95%CI= 1.8-8.1, p<0.001). Patients with intermediate Duke score had higher TREML4 mRNA levels than those with a low score (1.6-fold, p = 0.021). Patients with higher TREML4 mRNA levels were more prone to have increased Duke score (OR.=3.173, 95%CI= 1,29-7.78, p=0.012).

Conclusion: In conclusion, increased TREML4 mRNA expression in blood leukocytes is influenced by gene polymorphisms and it is associated with more risk of cardiovascular disease, suggesting its role as a potential biomarker to investigate atherosclerosis.

Tuesday, August 6, 2019

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

Clinical and Diagnostic Immunology

A-038

Implementation and Validation of Enzymatic Colorimetric Method for the Quantitative Analysis of Bile Acid

K. I. Softa, M. Sulaiman. *Clinical Chemistry Laboratories, Prince Sultan Military Medical City, Riyadh, Saudi Arabia*

Background: Bile Acids are formed in the liver from cholesterol, conjugated primarily to glycine and taurine, stored and concentrated in the gallbladder, and secreted into the intestine after the ingestion of a meal. Bile Acids are critical for digestion and absorption of fats and fat-soluble vitamins in the small intestine. Many waste products, including bilirubin, are eliminated from the body by secretion into bile and elimination in feces. An elevated fasting level, due to impaired hepatic clearance, is a sensitive indicator of liver disease. Serum Total Bile Acid (TBA) levels are used clinically as a sensitive and reliable index of hepatobiliary diseases. The Objective of this study to evaluate the performance of Randox TBA Reagent on Roche Cobas 8000 analyzer (c702 module) by enzymatic colorimetric method.

Method: TBA method validation was performed using Randox TBA Reagent on Cobas c702 module using serum or heparinized plasma samples. Method validation was done according to the laboratory policy followed CLSI guidelines (EP05-A3/EP06-A/EP09-A3/EP17-A2). Assay Principle: in the presence of Thio-NAD, the enzyme 3-alpha hydroxysteroid dehydrogenase (3-α HSD) converts bile acid to 3-keto steroids and Thio-NADH. The reaction is reversible and 3-α HSD can convert 3-keto steroids and Thio-NADH to bile Acids and Thio-NAD. In the presence of access NADH, the enzyme cycling occurs efficiently and the rate of formation of Thio-NADH is determined by measuring specific change of absorbance at 405nm. Precision study was performed using 60 quality control samples of 2 different concentration in inter run for a period of 5 days. Mean, SD and CV% were calculated and compared to the manufacturer recommendation. Method comparison study was done comparing 22 samples with proficiency testing RIQAS-peer group and Bioscientia reference lab. Linearity study was done using 8 different concentrations patient samples that spanning the analytical measurement range (AMR) from 3.2 to 180 Umol/L. Sensitivity test was performed using 5 samples of 0.9% Saline of zero total bile acid (TBA) concentration. Reference Range study done using 25 healthy individuals samples to verify the manufactures recommended reference range (2.0 - 10.0 Umol/L).

Results: Between days precision study for low and high concentrations, CV% were 2.4 and 0.6 respectively. Method comparison acceptable criteria slope 0.9 - 1.1 and correlation coefficient (r) > 0.97, data was plotted on scatter plot, the yield slope was 0.905 and correlation coefficient (r) = 0.970.The method was found linear over the AMR of 3.2 - 180.0 Umol/L. The limit of quantitation observed less than 3.2 Umol/L which is agreed with the manufacturer claim.

Conclusion: Overall performance of total bile acid (TBA) on Roche Cobas 8000 (c702 module) was acceptable by routine patients testing. It provides reliable results for both male and female individuals.

A-040

Analysis of Computer Automated versus Manual Readings for Monoclonal Immunoglobulin Concentration in Serum Protein Electrophoresis

A. Clavijo[1], N. Ryan[1], H. Xu[2], G. Singh[1]. [1]*Augusta University - Department of Pathology, Augusta, GA,* [2]*Augusta University - Department of Biostatistics and Epidemiology, Augusta, GA*

Background: Distinction between monoclonal gammopathy of undetermined significance, smoldering multiple myeloma and plasma cell/multiple myeloma is based partly on serum concentration of monoclonal immunoglobulin (MIg). MIg level represents tumor burden. Furthermore, progression of disease and effects of treatment are monitored by measuring the concentration of MIg. Methods to quantify MIg include, vertical drop method, tangent skimming, and peak deconvolution. Vertical drop method is most commonly used and may be affected by variation among individual personnel. To mitigate the variations among observers, Helena SPIFE densitometric scan uses a computer-based estimation of MIg peak. The purpose of our study was to determine the accuracy of the computer's estimation versus results by humans.

Methods: We retrospectively examined 300 SPEP gels with a single monoclonal peak in the gamma region. Gels with biclonal/oligoclonal patterns or peaks in the beta or alpha regions were excluded. Data collected were concentration of the MIg peak estimated by: (1) computer, (2) technologist (four technologists counted as a single group), and (3 and 4) independently by two residents. Paired, two tailed, T-test was used to compare the estimations between groups. Repeated measures ANOVA was also used to compare the estimations by humans. Regression analysis of the computer-generated readings versus mean readings of the three human groups was conducted. The data for the 300 readings were further divided into three equal groups of low, mid and high MIg levels, and compared as above

Results: In all 300 observations, the computer reading was higher than that by humans. For humans, the range of MIg peak concentration was 0.04 g/dL to 8.85 g/dL. Computer readings ranged from 0.11 g/dL to 9.1 g/dL. The mean values of technologists, resident 1, resident 2, average of human readings, and computer readings were 1.01, 0.90, 0.86, 0.92, and 1.21 g/dL. T-test and analysis of variance showed no difference among humans. However, when compared to computer estimations, every human group, and the average of all human groups, had a significantly lower reading. Regression analysis slope between humans and computer was 0.977 but the intercept was 0.19 g/dL representing a positive bias by computer. Overall, the computer reading was 32% higher than the average human reading. In the tertile groups, computer readings were higher by 87% in the lower, 48% in the middle, and 19% in the high tertiles.

Conclusion: The computer software has a consistent positive bias when quantifying the MIg peak concentration. This bias was seen in all 300 samples and had the largest difference from human readings at lower MIg concentrations. There was variability among the human groups but these differences were not significant. However, all three human groups showed a significantly lower reading than the computer estimation. While there is no gold standard for the quantification of MIg peaks, accurate readings are necessary to distinguish between the monoclonal gammopathic disorders, establish an estimate of tumor burden and monitor course of disease. We recommend that the computer algorithm be modified to provide MIg peak values consistent with human estimates to generate a more accurate result.

A-041

Highly Stable Nontoxic CuInZnS/ZnS//ZnS Quantum Dots for Quantitative Immunoassay Application

R. Wu, H. Shen, L. Li. *Henan University, Kaifeng, China*

Background: Due to the excellent optical characteristics, inorganic quantum dots (QDs) are ideal fluorescent probes for advanced biosensor, cellular imaging, cell labeling, gene sequencing, and chemical analysis. However, Most recent reports reveal that the applications in biological field not only require QDs to be strongly emitting, but also have low toxicity (free of heavy metal cations, such as cadmium, lead, and mercury). Here, we synthesized nontoxic CIZS/ZnS//ZnS QDs with higher stability and high photoluminescence quantum yields (PL QYs), and the CIZS/ZnS//ZnS QDs were successfully applied in quantitative fluorescent labeled lateral flow immunoassay (LFIA).

Methods: The thick shell CIZS/ZnS//ZnS QDs with on average PL QYs of up to 77% were synthesized. Then, the QDs transferred to aqueous solution by encapsulating with an amphiphilic polymer (polymaleic acid n-hexadecanol ester, PMAH). The aqueous CIZS core/shell QDs with high PL QYs (~58%) were conjugated with CRP antibodies, which were used as the fluorescence porbe for LFIA. To prepare QD based-LFIA strips, the treated sample pad were also chosen as conjugation pad, and on end of which sprayed with QD fluorescence probe, and the selected antibody and goat anti-mouse IgG were dispended onto the NC membrane to form the test line and the control line respectively.). Then, different concentrations of the human CRP standard samples were added to the end of the sample pad. At last, the fluorescence intensity of T line and C line on the LIFA strip were detected using PTI fluorescence measurement system (TimeMaster 400 USA).

Results: The as-prepared nontoxic CIZS/ZnS//ZnS QDs have on average PL QYs of up to 77% and PL QYs was still well maintained in aqueous solutions after phase transfer (up to 58%). Thick ZnS shell (~3.1 nm) around the surface of the core not only enhanced the PLQYs of the QDs, but also it provided better stability of hydrophilic thick shell CIZS core/shell//shell QDs in different environment. The highly stable nontoxic CIZS/ZnS//ZnS QDs were successfully applied in quantitative detection of CRP by LFIA, which showed a broad detection range (0-800 ng/mL) and low limit of detection (5.8 ng/mL).

Conclusion: Nontoxic CIZS/ZnS//ZnS core/shell QDs with higher stability and high PL QYs have been synthesized and adopted as fluorescence label into lateral flow im-

munoassay for quantitative detection of CRP. The limit of detection was much lower than that of most commercial CRP Point-of-care testing (POCT) product and was almost as low as that of cadmium-based quantum dot lateral flow test strip. Therefore, this may bring us a new approach by using nontoxic thick shell QDs as probes and indeed to achieve the ultra-sensitive detection, and the further development of this material has immense potential for future convenient and cost-effective *in vitro* nano-medical diagnostic kits.

A-042

Evaluation of Salivary Acetylcholine Level Measurement as a Marker of Dementia at Community Level

H. S. Chaudhury[1], S. Khandker[2], S. M. K. Ali[2]. *[1]International Medical College, Gazipur, Bangladesh, [2]Daffodil International University, Dhaka, Bangladesh*

Background: Dementia is a progressive debilitating condition having severe social and economic consequences. In Alzheimer's disease enzyme choline acetyltransferase (CAT) is less active than in an non-Alzheimer's brain resulting in a reduction in the synthesis of Acetylcholine. Salivary acetyl choline level is a promising marker of biochemical prediction and detection of subjects with Alzheimer's Disease and Senile Dementia with Community level applicability. The study is designed to measure Acetylcholine concentration in saliva of respondents with and without dementia. **Methods:** In this cross sectional analytical study 120 subjects (60 cases, and 60 age- and sex-matched controls) were selected from 374 respondents in an open sensitization Seminar. Respondents were above 60 years and below 85 years age. Dementia related information was collected based on Simon Fraser (SF)-36 scale. Controls were selected matching the age of cases having no symptoms of dementia. Structured questionnaire was used that included all variables of interest was used for data collection. Salivary acetylcholine level was measured by ELISA method and plasma glucose was measured by photometry (glucose peroxidase) method. **Results:** Dementia was more in males (60%) than in females (40%). The mean (±SD) fasting plasma glucose level was higher in Dementia subjects compared to control subjects (7.8±2.5 vs 6.2±1.2 mmol/L, P<0.001). The mean (±SD) salivary acetyl choline level was lower in Dementia subjects compared to control subjects (154±98 vs 412±113 pg/mL, P<0.001). **Conclusion:** Salivary acetyl choline level is lower in subjects with dementia and it is a useful indicator for biochemical screening of subjects at community level for Alzheimer's Disease and Senile Dementia.

A-043

Case Report: Gamma Heavy Chain Disease Associated with T Large Granular Lymphocytic Leukemia

P. Li, L. Qiu, W. Su, J. Han, J. Zhang. *Department of Clinical Laboratory, Peking Union Medical College Hospital, PUMC, beijing, China*

Background: Gamma heavy chain disease (gHCD) is a rare lymphoproliferative disorder characterized by the production of a truncated immunoglobulin heavy chain. Fewer than 200 cases have been reported in the literature. gHCD is associated with a variety of underlying lymphoproliferative disorders.Large granular lymphocyte (LGL) leukemia is another rare lymphoproliferative disorder.It is rare that two kind of rare disease happened in the same patient. **Methods:** The aim of this report is to show an rare case of gamma heavy chain disease in a 31 year old female patient with large granular lymphocytic leukemia focusing on the laboratory presentation. The gel electrophoresis,capillary electrophoresis, immunotyping ,blood smear, bone marrow TCR gene rearrangements and phenotype can aid in the diagnosis of this case. **Results:** The clinical, biochemical, haematological, and histological findings help to diagnose gamma heavy chain disease associated with T large granular lymphocytic leukemia. **Conclusion:** gHCD and LGL leukemia share some similar clinical features. It could result in a diagnostic dilemma.The pathogenesis of gHCD and LGL currently remains unclear,however, the different diagnostic method may contribute to a clearer understanding of its clinicopathological features.

A-044

Development of an Enzyme-Linked Immunosorbent Assay for Therapeutic Drug Monitoring of Ustekinumab

K. Farrag[1], M. Rohlfs[2], J. Ruppert[2], A. Eichhorn[2], F. Armbruster[2], J. Stein[3]. *[1]DGD Clinics Sachsenhausen, Frankfurt am Main, Germany, [2]Immundiagnostik AG, Bensheim, Germany, [3]Interdisciplinary Crohn Colitis Center Rhein-Main, Frankfurt/Main, Germany*

Background: Ustekinumab is a monoclonal therapeutic antibody against the p40 subunit of interleukin-12 and interleukin-23 approved for use in moderate to severe Crohn´s Disease (CD). Analysis of data from the phase 3 induction trials UNITI-1 and UNITI-2 demonstrated a significant exposure-response relationship of ustekinumab in CD. Interindividual differences in response to ustekinumab treatment may be explained in part by interindividual variability in pharmacokinetics. The aim of this work was to develop and validate an enzyme-linked immunosorbent assay (ELISA) using specific monoclonal antibodies to measure ustekinumab drug concentrations.

Methods: Samples diluted at 1:200 were added to microtiter plates coated with recombinant human monoclonal antibodies against ustekinumab for binding. Mouse anti-human immunoglobulin G1 (HRP-anti h IgG1) was used to detect bound ustekinumab. Assay performance characteristics were determined according to the European *in-vitro* diagnostic devices directive 98/79/EC.

Results: In both serum and plasma, the method was demonstrated to be linear from 1.10 to 37.35 ng/mL, showing non-linear behavior of less than ± 20 % in this interval. The limit of quantification (LoQ) for ustekinumab measurement in human serum samples was 0.953 ng/mL. Intra-assay variation (repeatability) was ≤ 9.5% (n=23), while inter-assay variation (reproducibility) was ≤ 9.1% (n=20). Linearity testing was performed by analyzing three serially-diluted samples spiked with ustekinumab; ustekinumab concentrations measured by the new assay were within 97% - 117% of the expected concentrations. The assay detected no false-positive signals from the samples of untreated patients. The specificity of the antibody was tested by measuring the cross-reactivity against a range of compounds with structural similarity to ustekinumab. There was no cross-reactivity observed.

Conclusion: This further improved ELISA offers a fast and accurate test with reproducible results. This assay has potential utility in therapeutic drug monitoring of patients receiving ustekinumab, and additionally in pharmacokinetic/pharmacodynamic studies of the drug.

A-046

Myositis Panel Clinical Usefulness in a Case of Histopathological Confirmed Necrotizing Autoimmune Myopathy (NAM) Diagnosis by Laboratory Methods

J. Diks, D. Daniel, A. Zuretti. *Suny Downstate Medical center, Brooklyn, NY*

Background: Necrotizing autoimmune myopathy (NAM) is a rare subgroup of idiopathic inflammatory myopathies probably accounts for 20% of Autoimmune myopathies (AIM). Mainly affects adults from 30 to 70 years of age in reported cases, most frequently women. Patients present with a subacute severe symmetrical proximal myopathy leading to muscle atrophy, associated with a markedly elevated creatine kinase level more than 10 times above the upper limit of normal at the time of onset of muscle weakness. Despite diverse causes, have the common histopathological features of myocyte necrosis without significant inflammation, unlike other inflammatory myopathies. These are most likely immune-mediated, as they respond to immunotherapy. The course is often severe but may be self-limiting and recovery may occur within weeks to months of discontinuing the causative agent, if identified. Anti-SRP and anti-HMGCoAR autoantibodies are frequently associated with this condition. Currently, seronegative NAM represents 20-30% of the cases. The objective of this study is to evaluate Myositis Panel after a confirmed histopathological muscle biopsy is performed.

Methods: A 30 YO F with necrotizing myositis was referred to Downstate Rheumatology for continued management. She had been diagnosed one year ago. She has outside state muscle biopsy, confirming necrotizing myopathy on H&E slides. Her disease had been in remission on IVIG and Rituxan until Feb 2018, at which she flared after tapering off prednisone. At that time, her prednisone was resumed and Rituxan was infused. She has had sustained resolution of her muscle spasm since receiving Rituxan with improvement of her muscle enzymes. Azathioprine was added to her regimen at last visit; she is tolerating this medication well. Hepatitis B, C serology, QuantiFeron, Myositis Panel, and serial CK levels were performed. Myositis Panel is a test that detects serum markers for myositis using Western Blotting, which includes

anti-HMGCoAR autoantibodies, Anti-Jo 1 antibody, EJ antibody, RNP, Mi-2, Ku, Signal Recognition Particle (SRP), Mi-2, PL-7, PL-12, and OJ antibodies.

Results: Serial CK levels showed correlation increase at times of flaring up to 1076 u/L [30-223 u/L]. Hepatitis B, C serology and QuantiFeron were unremarkable. All serological markers of Myositis Panel were negative.

Conclusion: Diagnosis of Necrotizing autoimmune myopathy is based on the clinical picture and on muscle biopsy showing minimal or no inflammatory infiltrates and marked muscle necrosis, associated with a markedly elevated creatine kinase level. These two autoantibodies Anti-SRP and anti-HMGCoAR are strongly associated in two-third of cases. Except for these two autoantibodies other serological markers of Myositis Panel like Anti-Jo 1 antibody, EJ antibody, RNP, Mi-2, Ku, Mi-2, PL-7, PL-12, and OJ antibodies are relatively useful in diagnosis and not useful in changing the plan of management. NAM pathophysiology is still poorly understood, notably because of a lack of animal models. Both anti-SRP and anti-HMGCR aAbs target intracellular proteins and the question of how they may reach their cognate antigen remains undetermined.

A-047

Biotin Sulfoxide is an Interferant in Several Roche Cobas e411 Immunoassays

J. Yang[1], J. R. Wiencek[2]. *[1]Medical Laboratories, University of Virginia, Charlottesville, VA, [2]Department of Pathology, University of Virginia, Charlottesville, VA*

Background: Biotin is a well-characterized interference in immunoassays utilizing biotin-streptavidin interactions. Biotin sulfoxide and bisnorbiotin are two major biotin metabolites that are detectable following acute or chronic biotin ingestion. Recent investigations using streptavidin microparticles to assess biotin interference showed that bisnorbiotin does not bind to streptavidin with the same affinity as biotin. From this study biotin sulfoxide was suspected to be the major biotin metabolite interference in biotin-streptavidin based immunoassays.

Objective: To determine the effects of biotin sulfoxide on several commonly measured analytes on the Roche Cobas e411 analyzer.

Methods : The effect of biotin sulfoxide interference on several Roche Cobas e411 immunoassays was assessed as recommended by the CLSI EP7-A2 clinical laboratory guideline. Three pools of plasma or serum were collected from healthy volunteers or patients diagnosed with a pituitary adenoma and were told to avoid biotin or multivitamin supplement one week before collection. For comparison biotin sulfoxide or biotin was titrated into paired pools with increasing concentrations of interferant to achieve final concentration of 0, 0.06, 0.18, 0.54, 1.08, 2.16, 3.24 and 4.32 μmol/L (equivalent to 0, 14.7, 44.0, 131.9, 263.9, 527.7, 791.6, 1055.4 ng/mL of biotin). These samples were then analyzed for adrenocorticotropic hormone (ACTH), growth hormone (hGH), C-peptide, antibodies to thyroid peroxidase (Anti-TPO), antibodies to thyroglobulin (Anti-Tg) or dehydroepiandrodrosterone sulfate (DHEA-S).

Results: Biotin sulfoxide falsely increased results of competitive immunoassays (DHEA-S, Anti-Tg and Anti-TPO) and falsely decreased results of sandwich immunoassays (C-peptide, ACTH and hGH). The results demonstrate that 2 to 3 fold amount of biotin sulfoxide is needed to have the same effect of biotin within the studied concentration range 0.06-4.32 μmol/L (Figure 1).

Conclusion: Metabolites of known interferants should be considered as additional sources of interference in clinical laboratory assays. Biotin sulfoxide at specific concentrations was identified as an interferant in all immunoassays investigated in this study.

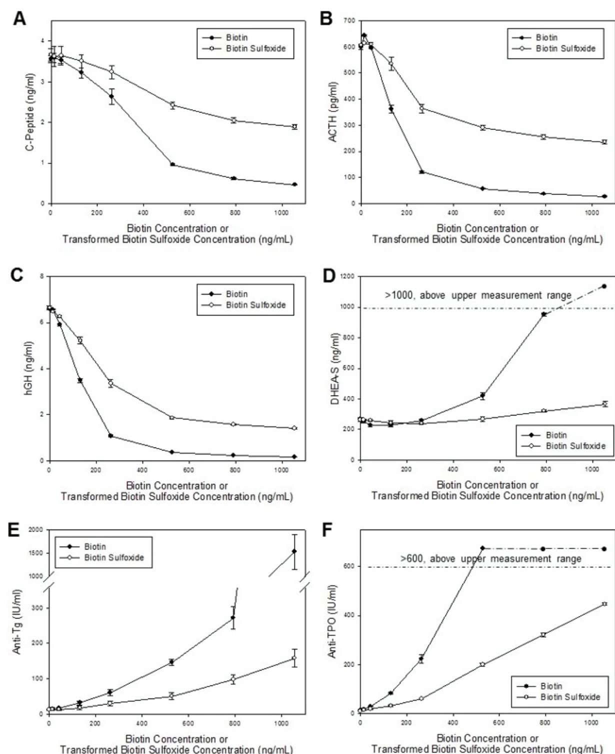


Figure 1. Biotin and biotin sulfoxide falsely decreased the results of sandwich immunoassays C-peptide (A), ACTH (B) and hGH (C), but falsely increased the results of competitive immunoassays DHEA-S (D), Anti-Tg (E) and Anti-TPO (F). Biotin and biotin sulfoxide were spiked into samples at the same molar concentrations. Transformed biotin sulfoxide concentration means the biotin required to be metabolized into biotin sulfoxide. Dose response curves were plotted for both biotin (●) and biotin sulfoxide (○). Experiments were repeated 3 times. Error bars represent SEM (standard error of the mean).

A-048

A New Direct Enzymatic Assay for the Determination of Beta-hydroxybutyrate on Chemistry Analyzer Platforms

M. Grimmler, B. Cucu, K. Eulberg, A. Lein, H. Müller. *DiaSys, Holzheim, Germany*

Background: Determination of β-hydroxybutyrate in serum or plasma can be used for the diagnosis and prognosis of diabetic ketoacidosis, alcoholic ketoacidosis and hypoglycemia. Recently the determination of β-hydroxybutyrate is used to detect neurodegenerative diseases, inhibition of adipocyte lipolysis or tumor progression. Identification of ketones [β-hydroxybutyrate (78%), acetone (2%) and acetoacetat (20%)] in serum is exempt from FDA 510(k) premarket notification procedures. The objective of this study was to evaluate CLSI performance of DiaSys β-Hydroxybutyrate 21 FS reagent on Architect c8000™ clinical chemistry analyzer.

Methods: DiaSys β-hydroxybutyrate assay, β-Hydroxybutyrate 21 FS, is a liquid stable ready-to-use 2-component reagent based on a UV test principle which utilizes the β-hydroxybutyrate-dehydrogenase dependent conversion of NAD to NADH. The rate of NADH formation is determined by measuring a specific change of absorbance at 340/700 nm, which is directly proportional to the concentration of β-hydroxybutyrate in sample material. Comparative studies were performed on Architect c8000™ with 147 serum samples according to CLSI protocol [EP5]. Data have been evaluated by using regression analysis according to Passing and Bablok.

Results: The performance evaluation on Architect c8000™ revealed a linearity given from 0.05 mmol/L up to 6 mmol/L [Fig.1]. The reagent shows good correlation for sera in a comparison study with a commercially available enzymatic colorimetric assay [r=0.9991; Passing/Bablok: y= 1.012 X + 0.001 mmol/L] [Fig.2]. Moreover, DiaSys β-Hydroxybutyrate 21 FS is highly precise with a total precision according to CLSI protocol [EP15-A2] ≤ 1.67 % [Fig. 3].

Conclusion: DiaSys ß-Hydroxybutyrate 21 FS is adaptable on Architect c8000™ and shows outstanding performance especially for linearity and precision. The assay meets the requirements for suitable diagnosis and monitoring of patients with diabetes

to prevent ketoacidosis. The performance of the test is highly competitive in comparison to already available products in the market.

A-049

Anti-Brush Border Antibodies in a Patient Coinfected with *Mycoplasma pneumoniae* and the Epstein-Barr virus: A Case Report

K. Kim[1], L. Jearn[2], T. Kim[2]. [1]*Department of Diagnostic Immunology/Laboratory Medicine, Seegene Medical Foundation, Seoul, Korea, Republic of,* [2]*Department of Diagnostic Immunology/Laboratory Medicine, Hanyang University Medical Center, Seoul, Korea, Republic of*

Anti-brush border antibodies (ABBA) have been shown to react with brush border membrane antigens in the periluminal area of proximal tubular cells in rat kidney tissues. However, the clinical significance of ABBA remains unclear to date. A 17-year-old male with general myalgia, fever, jaundice, and cough displayed elevated levels of bilirubin, aspartate aminotransferase **(**AST), and alanine aminotransferase **(**ALT) during primary care. The patient was admitted because of acute hepatitis suspicion. Laboratory tests revealed elevated levels of alkaline phosphatase (141 U/L), lactate dehydrogenase (708 U/L), and AST/ALT (51/63 U/L). However, viral hepatitis tests were normal. While the antimitochondrial antibody (AMA) and anti-liver/kidney microsome-1 (LKM-1) antibody tests were negative, the anti-smooth muscle antibody (ASMA) test was weakly positive by indirect immunofluorescence using three types of rat tissues (stomach, kidney, and liver). During the process, the kidney tubules were partially immunopositive in the periluminal area, resulting in the incidental detection of ABBA. Next, we evaluated the ABBA titer value (1:640). The total bilirubin/direct bilirubin level was 7.6/2.0 mg/dL. The findings of the direct/indirect antiglobulin and anti-C3d tests were 3+/1+ and 3+, respectively. Accordingly, the patient was suspected to present hemolytic anemia. The serum anti-mycoplasma antibody test suggested a positive titer of 1:320, and the IgM test, monitoring infection with the Epstein-Barr virus (EBV), was positive. Finally, the patient was diagnosed with autoimmune hemolytic anemia caused by *Mycoplasma pneumoniae* and EBV infection and treated with antibiotics. The presence of ABBA was confirmed in the serum of a patient with autoimmune hemolytic anemia coinfected with *M. pneumoniae* and EBV. Although the presence of ABBA has been occasionally reported, no case of coinfection exists in the literature. Infections prompt a lack of auto-tolerance, causing confusions in the recognition of microbial antigens and self-antigens and producing various autoantibodies. A coinfection could lead to the acquired immunodeficient state of the host, possibly stimulating the production of autoantibodies. ABBA is a rare antibody that can be detected when testing tissues for AMA, LKM-1, and ASMA; hence, judicious probing for the presence of ABBA could yield more such cases, facilitating the determination of its clinical significance. Further, ABBA may be introduced as a routine test when testing AMA, LKM-1, and ASMA.

A-050

The Diagnostic Value of Anti-cmDNA Combined with ANA and Anti-dsDNA in Systemic Lupus Erythematosus

Y. Huang, W. Wang, K. Chen, D. Wen. *Sun Yat-sen University affiliated Zhongshan hospital, Zhongshan, China*

Background: Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease in which the immune system attacks the patient's tissues, resulting in inflammation and damage. Early diagnosis and treatment is the key process to improving both the disease prognosis and the patient's quality of life. Autoantibody formation commonly occurs in SLE, including anti-nuclear antigen antibodies(ANA) and anti-double strand DNA antibodies(anti-dsDNA), which have been widely used in diagnosing SLE clinically. However, ANA has a poor specificity and anti-dsDNA has a poor sensitivity in the diagnosis of SLE, a supplementary detective marker was critical in the early diagnosis of the disease. Previous study has shown that a new marker, anti-cell membrane-associated DNA antibodies (anti-cmDNA) can serve as a specific marker in SLE patients. To date, studies on the diagnostic value of these antibodies in SLE have been limited. This study was aimed to compare the diagnostic value of anti-cmDNA, ANA, and anti-dsDNA separately and assembly, and further evaluated the significance of combined detection of these markers in SLE.

Methods: The current study was constructed at Department of Laboratory medicine, Zhongshan Hospital of Sun Yat-sen University. 101 patients with SLE, 94 patients with other rheumatic diseases and 78 healthy volunteers were recruited from September 2017 to December 2017. ANA was detected by indirect immunofluorescence assay. Anti-cmDNA and anti-dsDNA were both detected by enzyme linked immunosorbent assay(ELISA). Statistical analyses including receiver operating curves (ROCs) and chi-square(χ2)test were applied in the study.

Results: The positive percentage of anti-cmDNA was higher in the SLE group, with the percentage of 68%. While, the positive percentages of the other rheumatic disease group and the healthy control group were 19% and 0%, respectively ($P<0.05$). When evaluating the diagnostic values of three mentioned antibodies separately, anti-cmDNA had the highest accuracy (82%), Youden's index(YI 0.58), and area under ROC curve(AUC 0.79).While using combined makers, anti-dsDNA /anti-cmDNA, ANA+(anti-dsDNA/anti-cmDNA), anti-dsDNA/(ANA+anti-cmDNA), anti-cmDNA /(ANA+anti-dsDNA) and (ANA+anti-cmDNA)/(anti-dsDNA+anti-cmDNA)/(ANA+anti-dsDNA)all have the highest accuracy(84%), YI(0.65) and AUC(0.83).

Conclusion: Anti-cmDNA was the vital marker for the diagnosis of SLE with higher accuracy compared with ANA and anti-dsDNA . Further, the combined detection of the three mentioned antibodies was crucial in the early diagnosis of SLE, resulting in improving both the disease prognosis and the patient's quality of life.

A-051

Performance Evaluation of an Homogeneous Cholylglycine Immunoassay in a Clinical Chemistry Laboratory

J. QI, L. Xiao, J. Gong. *Beijing Strong Biotechnologies. Inc, malden, MA*

Background: Serum Cholylglycine (CG) is a combination of secondary bile acids conjugated with glycine. Recently CG has become a sensitive biomarker for diagnosis of liver disease. The main purpose of this study was to evaluate the performance of an enzyme multiplied immunoassay technique (EMIT) CG kit. **Methods:** The EMIT CG kit was developed by Beijing Strong Biotechnologies , Inc. A Hitachi 7180 clinical chemistry analyzer (Made in Japan) was used for all the following studies, with a calibration cycle of 7 days. Tri-level controls and 2 clinical sample pools with different CG concentrations were tested in replicates of 21 to calculate the repeatability. The precision studies were evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP05-A2. Tri-level controls were tested in replicate in two runs for a minimum of 20 days. Linearity and spike recovery studies were evaluated according to EP6-A2 and EP15-A2, separately. CG from Sigma (HPLC grade, purity≥97%) was dissolved with alcohol to prepare a 10mg/ml stock solution, then the stock solution was diluted into the clinical serum pools with final concentrations shown in Fig 1C. Interference studies were measured as the ratio of the response value and the expected concentration of different CG analogs. **Results:** The repeatability, precision, spike recovery, and interference data are demonstrated in Table 1A, 1B, 1C and 1D. The linear range of CG kit is 0.6 to 40 µg/ml, and the linear equation is $y = 3.9813x - 2.7977$ with $R^2 = 0.9982$. **Conclusion:** The CG kit has a good performance in repeatability, precision, spike recovery, interference and linearity which can provide a better option for clinical cholylglycine detection.

Table 1A: Repeatablity data of CG kit. Tri-level controls and 2 clincial sample pools were measured in deplicate of 21.

Sample	Target (μg/ml)	Mean (μg/ml)	STD	CV
Control 1	1.00	1.15	0.03	2.60
Control 2	2.50	2.55	0.06	2.18
Control 3	10.00	10.23	0.11	1.09
sample 1	20.00	18.54	0.32	1.74
sample 2	35.00	34.40	0.65	1.90

*STD stands for standard deviation, CV stands for Varible coefficient.

Table 1B: Precision data of the CG kit. 3 controls were tested in replicate in two runs (interval for 2 hours at least) per day for a minimum of 20 days.

Sample	Mean (μg/ml)	Within Run SD	Within Run CV	Total Run SD	Tatal Run CV
Control 1	0.92	0.01	1.47%	0.04	4.19%
Control 2	2.52	0.04	1.44%	0.08	3.37%
Control 3	10.07	0.14	1.36%	0.22	2.21%

Table 1C : Spike recovery data of the CG kit. 10mg/ml stock solution was prepared with CG powder, and diluted to different concentrations with clinical serum pool. Each sample were tested 3 times and bias were calculated between target and mean recovery value.

sample	Target (μg/ml)	Recovery (μg/ml)	Bias
sample 1	0.5	0.47	-6.00%
sample 2	1.2	1.15	-4.17%
sample 3	2.5	2.45	-2.00%
sample 5	10	10.24	2.40%
sample 6	20	18.54	-7.30%
sample 7	35	34.4	-1.71%

Table 1D : Interference studies show CG kit has a low recognition rate with the CG analogs up to the concentration list below.

Interference Substence	Expected Concentration (μg/ml)	Response Value (μg/ml)	Interference Rate
Glycocholic acid	80	2.53	3.16%
Glycochenodeoxycholic acid	80	0.27	0.34%
Chenodeoxycholic acid	80	0.07	0.09%
Ursodesoxycholic acid	80	0.04	0.05%
Sodium cholic acid	80	5.15	6.44%
Sodium deoxycholic acid	80	0.11	0.14%

A-053

Urinary Adiponectin as a Biomarker for Chronic Kidney Disease due to Diabetic Nephropathy

Y. Jiang[1], K. Saito[1], K. Nakaishi[1], S. Watabe[1], M. Inada[2], T. Yoshimura[3], E. Ito[2]. *[1]TAUNS Laboratories,Inc., Izunokuni,Shizuoka, Japan, [2]Waseda University, Tokyo, Japan, [3]Health Sciences University of Hokkaido, Hokkaido, Japan*

Background: The chronic kidney disease (CKD) is widely diagnosed on the basis of albuminuria and the glomerular filtration rate (GFR). A more precise diagnosis of CKD, however, requires the assessment of other factors. Urinary adiponectin recently attracted attention for CKD assessment, but evaluation is difficult due to the very low concentration of urinary adiponectin in normal subjects.

Methods: We developed an ultrasensitive ELISA coupled with thio-NAD cycling to detect trace amounts of proteins, which allows us to measure urinary adiponectin at the subattomole level. We measured urinary adiponectin levels in 59 patients with diabetes mellitus (DM) and 24 subjects without DM (normal) to test our hypothesis that urinary adiponectin levels increase with progression of CKD due to DM.

Results: The urinary adiponectin levels were 14.88 ± 3.16 (ng/mg creatinine, mean ± SEM) for DM patients, and 3.06 ± 0.33 (ng/mg creatinine) for normal subjects. The threshold between them was 4.0 ng/mg creatinine. The urinary adiponectin levels increased with an increase in the CKD risk (Kruskal-Wallis test, $P < 0.01$). Furthermore, urinary adiponectin mainly formed a medium-molecular weight multimer (a hexamer) in DM patients, whereas it formed only a low-molecular weight multimer (a trimer) in normal subjects. That is, the increase in urinary adiponectin in DM patients led to the emergence of a medium-molecular weight form in urine. On the other hand, we investigated whether urinary adiponectin levels change after exercise. Urine was collected from three normal subjects before and after performing anaerobic exercise and aerobic exercise. The urinary adiponectin levels were significantly higher after anaerobic exercise than before it (paired *t*-test, $P < 0.05$), whereas there was no significant difference between before and after aerobic exercise (paired *t*-test, $P > 0.05$). Thus, even in normal subjects, urinary adiponectin levels exceed the threshold of 4.0 ng/mg creatinine after anaerobic exercise. As expected, low-molecular weight multimer bands strongly appeared in the western blotting of the normal subject urine samples after anaerobic exercise. DM patients, however, are not likely to have performed anaerobic exercise before going to the hospital.

Conclusion: We developed an ultrasensitive ELISA, and measured urinary adiponectin levels in DM patients and normal subjects. The urinary adiponectin levels in DM patients were higher than normal subjects. The urinary adiponectin levels increased with an increase in the CKD risk. Urinary adiponectin formed medium- and high-molecular weight multimers in DM patients, whereas it formed only a low-molecular weight multimer in normal subjects. Our new assay showed that urinary adiponectin could be a new diagnostic index for CKD. This assay is a noninvasive test using only urine, thus reducing the patient burden.

A-054

Analysis of Aging Characteristics of Peripheral T Lymphocyte Subsets in Healthy Population in Western China

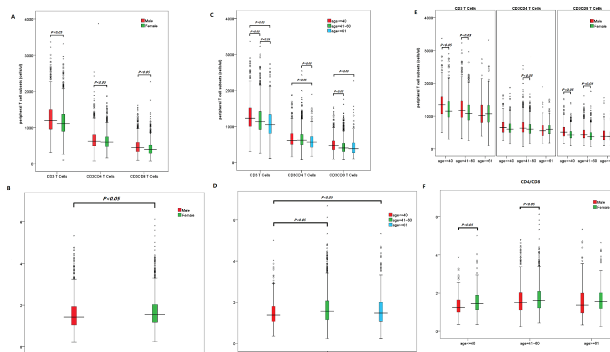
G. Zhang, B. Cai, W. Feng, Y. Li, L. Yan, H. Xu. *Department of Laboratory Medicine, West China Hospital of Sichuan University, Chengdu, China*

Objective: To explore the age and gender characteristics of peripheral T lymphocytes subsets and establish its specific reference in Western-Chinese Han population.

Methods: Retrospectively analyse the expression of peripheral T lymphocyte subsets in 3,049 healthy Western-Chinese Han population in our hospital from April to June 2018. Absolute count of T lymphocyte was detected by flow cytometre on single platform.

Results: ①Expression characteristics of peripheral T lymphocyte subsets in male or female: The absolute counts of CD3, CD4, CD8 and CD4/CD8 ratio in male population were significantly higher than those in female population (Figure 1A and 1B). ② The aging characteristics of peripheral T lymphocyte subsets: CD3+, CD4+ and CD8+ T cell counts and CD4/CD8 ratio decreased with age, among which CD3+ and CD8+ T cells changed most obviously, CD4+ T cells in age>60y group showed a significant decrease (Figure 1C and 1D).③ Stratified with age and gender to analyse expression characteristics of peripheral T lymphocyte subsets: when age <40y and 40-60y, CD3+, CD4+, and CD8+ T cell numbers were significantly higher in male than in female; when age over 60y, the difference of T lymphocyte subsets absolute count between male and female disappeared. (Figure 1E and 1F)④ Establish a gender- and age-specific reference, which suggested that with aging the number of T lymphocyte subsets decreased, and in age over 60y peripheral T cells subsets counts in female were similar to those in male.

Conclusions: Peripheral T lymphocyte subsets in apparently healthy populations in Western China reflected the significant gender and age differences. When age below 60y, there were significantly increased peripheral T lymphocyte counts in male compared with that in female, with aging peripheral T lymphocyte counts gradually decreased, which suggested that lower immunity characterized by decreasing of T cells in elderly patients was one of the important factors leading to tumor or infectious diseases.



A-055

Disordered Intestinal Microbes are Associated with the Activity of Systemic Lupus Erythematosus

y. qiu. *Laboratory Medicine Center, Nanfang Hospital, Southern Medical University, Guangzhou, China*

Background: Intestinal dysbiosis is implicated in Systemic Lupus Erythematosus (SLE). However, the evidence of gut microbiome changes in SLE is limited, and the association of changed gut microbiome with the activity of SLE, as well as its functional relevance with SLE still remains unknown. **Methods:** Here, we sequenced 16S

rRNA amplicon on fecal samples from 40 SLE patients (19 active patients, 21 remissive patients), 20 disease controls (Rheumatoid Arthritis patients), and 22 healthy controls, and investigated the association of functional categories with taxonomic composition by Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt). **Results:** We demonstrated SLE patients, particularly those active patients, had significant dysbiosis in gut microbiota with reduced bacterial diversity and biased community constitutions. Among the disordered microbiota, the genera *Streptococcus*, *Campylobacter*, *Veillonella*, the species *Streptococcus. anginosus* and *Veillonella. dispar*, were positively correlated with lupus activity, while the genus *Bifidobacterium* was negatively associated with disease activity. PICRUSt analysis showed metabolic pathways were different between SLE and healthy controls, and also between active and remissive SLE patients. Moreover, we revealed that a random forest model could distinguish SLE from RA and healthy controls (AUC = 0.792), and another random forest model could well predict the activity of SLE patients (AUC = 0.811). **Conclusion:** In summary, SLE patients, especially the active patients, show an apparent dysbiosis in gut microbiota and its related metabolic pathways. Among the disordered microflora, 4 genera and 2 species are associated with lupus activity. Furthermore, the random forest models are able to diagnose SLE and predict disease activity.

A-056

Performance of the Enzymatic Alinity c Hemoglobin A1c Assay

M. Berman, L. Templin. *Abbott Labs, North Chicago, IL*

Background: Type 2 diabetes is a serious long-term condition that is due to insulin resistance of cells. Higher ambient glucose levels and longer red blood cell circulation time correlate with higher concentrations of Hemoglobin A1c (HbA1c). HbA1c measurements have been widely used in the monitoring of long-term blood glucose control and compliance in individuals with diabetes mellitus. An automated whole blood Hemoglobin A1c (HbA1c) test has been developed on the Alinity c system to detect the fraction of hemoglobin A that is glycated at one or both *N*-terminal valines of the *beta*-chain. This test automatically lyses the red blood cells and utilizes an enzymatic method that specifically measures *N*-terminal fructosyl dipeptides of the *beta*-chain of HbA1c. Two separate measurements are made: glycated hemoglobin (HbA1c) and total hemoglobin (THb) which are used to determine the percent HbA1c (National Glycohemoglobin Standardization Program [NGSP] units) or the hemoglobin fraction in mmol/mol (International Federation of Clinical Chemistry and Laboratory Medicine [IFCC] units).

Methods: Key performance testing including precision, limit of quantitation (LoQ), linearity, interference and method comparison were assessed per Clinical and Laboratory Standards Institute (CLSI) protocols. In addition, method comparison samples were assessed versus NGSP Secondary Reference Laboratory (SRL) method following CLSI EP09-A3 to verify standardization.

Results: Within-lab (total) imprecision for reported results in NGSP units ranged from 0.5% to 1.3 % CV. The assay is linear across the measuring interval of 4.0 to 14.0 % HbA1c. No interference was observed from similar compounds such as acetylated and carbamylated hemoglobin. Specificity of samples containing hemoglobin variants C, D, E, and S were within +/- 5% of results from an NGSP secondary reference laboratory. Only the hemoglobin variant F demonstrated a negative bias that was proportional to the concentration of the variant F in the sample. Correlation to the NGSP Secondary Reference Laboratory (SRL) method demonstrated a slope of 1.00, intercept of -0.10, correlation coefficient of 0.99, and a predicted bias of less than 3% at 6.0, 6.5, and 7.0 %HbA1c using Deming regression. An analysis of Allowable total difference (ATD) demonstrated > 99.3% of observations were in the ATD zone and the low limit of the two sided 95% confidence interval was 95.9%.

Conclusion: These results demonstrate that the Alinity c Hemoglobin A1c assay is a precise and accurate method for measuring HbA1c in human whole blood. The performance supports use of this assay as an aid to diagnose and monitor diabetes mellitus.

A-057

Increase of the Incidence of Hepatitis A Cases in the Metropolitan Region of Florianopolis between 2015 and 2018

J. Nunes, M. Balestrin, A. Lopes, D. Faria, M. Soika, D. Canali. *Laboratório Médico Santa Luzia, São José, Brazil*

Background: Hepatitis A is an acute infection caused by the Hepatitis A virus (HAV), which belongs to the *Picornaviridae* family. Its transmission occurs via the fecal-oral route through contaminated water and food or person-to-person contact. In recent years, transmission through sexual exposure among homosexual men has been reported in the literature. In this context, the present study aimed to evaluate the increased incidence of HAV cases in the region of the Metropolitan Region of Florianópolis.

Methods: The HAV IgM determinations were analyzed in blood samples between 2015 and 2018. When positive, the results were correlated with sex and age using a database of the Great Florianópolis.

Results: A total of 11217 tests were processed for the anti-HAV IgM antibody throughout the period evaluated. Among the reactive results, the incidence in 2015, 2016, 2017 and 2018 were 3 (0.23%), 2 (0.06%), 22 (0.67%) and 33 (0.93%) respectively, representing a total of 60 (0.53%) cases. Regarding sex, there were 5781 tests for females and 5436 for males. The results showed higher incidence of hepatitis A in males 48 (0.89%) than in females 12 (0.21%). Concerning age, the prevalence of the reactive results between 0 and 19 years, 20 and 39 years, 40 years or more were 2 (0.26%), 38 (0.81) and 20 (0.34%) cases, respectively. Moreover, in the age group between 20 and 39 years, age range with the highest concentration of positive results, 35 cases occurred in males.

Conclusion: An increased incidence of Hepatitis A was observed. In addition, in 2017 the cases increased three times more while in 2018 the cases number increased four times more when compared to the year 2015. The population with the highest incidence was male in the age group between 20 and 39 years. However, we can not affirm sexual exposure as the main route of transmission, since we do not have the data to prove this information.

References: 1. European Centre for Disease Prevention and Control. 2017 Hepatitis A outbreaks in the EU/EEA mostly affecting men who have sex with men. Stockholm: ECDC; first update, 23 February 2017.

2. Ministério da Saúde. Boletim Epidemiológico Hepatites Virais 2018**.** Brasília: Secretaria de Vigilância em Saúde - Ministério da Saúde; 2018. v. 49, n 31.

A-058

Evaluation of Procalcitonin Assay on the Abbott Architect i2000 Analyzer

K. Young Jae. *Samsung Changwon Hosp., Changwon, Korea, Republic of*

Background: Procalcitonin (PCT) is widely used as a diagnostic biomarker for bacterial infection and sepsis. As the number of test is expected to increase gradually in the coming years, it is highly desirable to shift our testing instruments into a high-speed, automated system to yield high throughput. The aim of this study is to assess the analytical performance of Brahms PCT reagents in the PCT assay by the Architect i2000 analyzer. **Methods:** PCT was quantitatively determined by the PCT assay on the Architect i2000 analyzer based on a two-step chemiluminescent microparticle immunoassay (CMIA). An evaluation was performed by following the procedure guidelines of Clinical and Laboratory Standard Institute (CLSI). We evaluated the basic QA including precision, linearity, carryover, and reference ranges. The accuracy of the method was also assessed by comparison test. A total of 35 samples obtained from the patients in our hospital have been tested with the μ-TAS immunoanalyzer (Wako, Japan), which has been in clinical use at our laboratory for the past five years. The obtained results were compared to those derived using the of Brahms PCT reagents on the Architect i2000 analyzer. **Results:** The within-run precision (%CV) values of the Architect i2000 analyzer, when tested in low, middle and high level controls (ng/mL: L1 (0.2), L2 (2.02), and L3 (71.4)) for PCT, were 2.2, 1.3, and 2.6%, respectively. Their counterparts for the between-run CVs were 2.5, 2.0, and 2.2%, respectively. The system exhibited a good linearity in the range of 0.00 to 99.2 ng/mL (calibration slope of 0.969 at zero offset) with the limit of detection as 0.02 ng/mL. It showed the carryover rate of 0.00 ng/mL. The reference range was verified as less than 0.05 ng/mL. According to the comparison of PCT results between Abbott Architect and μ-TAS, the two assay approaches exhibited a strong correlation (r >0.99: slope 0.945 and intercept 0.387). The mean bias between the two methods is 0.891 to imply that the Architect results are on average about 8% less than those of the μ-TAS immunoanalyzer

Conclusion: According to our study, the Brahams PCT assay based on Architect i2000 analyzer maintained enhanced performances (e.g., good linearity, precision, and no carryover). Although Abbott Architect showed a slightly reduced value compared to the μ-TAS immunoanalyzer, the PCT results of both systems exhibited a good correlation. The results of our study were successful to validate that Architect i2000 analyzer should be a highly suitable clinical analyzer for PCT assay.

A-059

Clinical Application Evaluation of a Fourth-generation HIV Antigen Antibody Combination Screening Assays

h. han, y. huang, q. dong, w. wang, k. chen. *Zhongshan hospital of Sun Yat-sen University, Zhongshan, China*

Background: Human immunodeficiency virus infection and acquired immunodeficiency syndrome (HIV/AIDS) is an infectious disease with high mortality. Its early diagnosis is crucial. The Elecsys HIV combi PT assay is a fourth generation HIV screening assay that combines HIV antibody and p24 antigen detection. This study examined the sensitivity and specificity of this method in the detection of patients in Zhongshan city, and evaluated the reliability of using signal-to-cutoff (S/CO) ratios to distinguish false positive HIV infection, and analyzed the causes of false positive results.

Methods: From Jan. 1, 2016, to Jun. 30, 2018, we conducted a fourth-generation HIV test of retrospective HIV screening samples at Zhongshan Hospital of Yat-sen University, and assessed the reliability of using signal-to-cutoff (S/CO) ratios to distinguish false positive HIV infections and analyzed false positives.

Results: A total of 111,556 samples were analyzed by the HIV combination PT assay, and 122 humans were identified as HIV-1 infection by immune-gold, western blot (WB) and HIV nucleic acid assays. The median S/Co ratio for HIV false positive specimens was 3.27, while the HIV-infected specimen was 391.7 ($P < 0.001$). Receiver of Operator Characteristic (ROC) analysis showed that the best diagnostic point for HIV was 22.85 S / CO. The sensitivity, specificity and Youden index were 100%, 97.8% and 0.978, respectively. The misdiagnosis rate was 2.2%, and the missed diagnosis rate was 0. The proportion of misdiagnosed specimens, 26.4%, was the highest in patients with malignant tumors and blood diseases.

Conclusion: The results of this study show that the fourth-generation Elecsys HIV combination PT test is sensitive and specific to HIV screening and can be a useful adjunct to guide clinicians in managing HIV infection. Our research data provides a reference for subsequent research and HIV testing in the region.

A-060

Comparison and Analysis of Clinical Diagnosis Performance in Grave's Disease of a Novel TSI Immunoassay versus an Automated TRAb Immunoassay: A Chinese Multicenter Study

C. c. Ma, L. Qiu, X. Cheng. *Peking Union Medical College Hospital Clinical Lab, Beijing, China*

Background: Thyroid-stimulating hormone receptor (TSHR) antibodies, generically known as TSH receptor antibodies (TRAb), are pathogenetic and diagnostic hallmarks of Graves' disease (GD). TRAb may mimic or block the action of thyroid-stimulating hormone (TSH) or be functionally neutral. At present, two different types of fully automated assays can detect the level of TRAb: thyroid-stimulating immunoglobulin (TSI) and TRAb immunoassays. The aim of this study was to evaluate the clinical diagnostic performance of these two assays., and their correlation and agreement.

Methods: Sera were evaluated from 1000 subjects from three centers representing a variety of conditions: 100 subjects with untreated GD, 200 with treated GD, 62 with autoimmune thyroid disease (AIT), 216 with other thyroid diseases, 214 with non-thyroid autoimmune diseases (NTAD), and 208 others (including 17 healthy controls and 191 subjects with other diseases). A TSI immunoassay was used on 1000 serum samples, while the TRAb immunoassay was used in parallel. The Thyretain TSI Reporter Bioassay was performed on 86 samples whose TSI results were inconsistent with the TRAb results. A human thyroid-stimulating blocking antibody (TSBAb) ELISA was used on TSI-negative and TRAb-positive samples in control groups (n = 52).

Results: When comparing untreated GD patients with the other four groups(AIT,NTAD,OTHER-T,OTHERS), the area under the curve for the TSI immunoassay was 0.991 (95% confidence interval (CI): 0.985-0.997), which was not inferior to that of the TRAb assay (0.987, 95% CI: 0.976-0.994) (P=0.4471). Compared to the TRAb immunoassay, the TSI immunoassay showed higher specificity (96.86% vs. 88.71%, P<0.0001), positive predictive value (PPV) (81.67% vs. 55.37%, P<0.0001), and positive likelihood ratio (LR+) (31.18 vs. 8.68), respectively. And the rest of the measures of accuracy were at least comparable to those of the automated TRAb immunoassays. The Spearman correlation coefficient was 0.758 (P<0.0001, 95% CI: 0.7294-0.7839) and intraclass correlation coefficient (ICC) was 0.773 (95% CI: 0.650-0.857, P<0.0001) in quantitative analysis. The Cramer's V coefficient was 0.812 (95% CI: 0.774-0.848), and the kappa coefficient was 0.806 (95% CI: 0.765-0.843) in qualitative analysis. The agreement rate for the TSI immunoassay with the bioassay was significantly higher than that of the automated TRAb immunoassay: (87.21% vs. 12.90%, P<0.0001).

Conclusion: The clinical diagnostic performance for Graves' disease of the TSI immunoassay is not inferior to that of TRAb immunoassay. And the TSI immunoassay demonstrates good correlation and agreement with the TRAb immunoassay. The TSI immunoassay could be a more accurate diagnostic method for GD than the TRAb immunoassay as we provided some evidence that the former is specific for TSI only.

A-061

Performance Evaluation of the Immunalysis Opioid Panel on the ARCHITECT c System from Abbott Laboratories

L. Ruvuna[1], K. Huynh[2], S. Martinez[2], G. Wang[2], M. Vincent[2], M. Moore[1]. [1]*Abbott Labs, Abbott Park, IL,* [2]*Immunalysis Corporation, Pomona, CA*

Background: With the continued over prescription of pain medication, opioid addiction has become an increasingly larger proportion of the drug abuse problem. This has led to a rapid rise in overdose deaths and a need for new opioids tests. The Immunalysis Opioid panel of assays are used to screen for the presence of abused drugs in human urine around a defined cutoff level. Qualitative applications provide results as positive or negative for the drug, while the semiquantitative applications provide an approximate concentration. Positive screening results on both applications must be confirmed by a more specific analytical method such as GC/MS or LC-MS/MS. The Immunalysis Opioid panel of assays provide accurate, reliable results for a wide range of opioids with high specificity and low cross-reactivity. The rapid turnaround time at lower costs on the ARCHITECT c systems assures results are provided to the clinicians quickly and prevent delay in treatment.

Objective: To demonstrate the analytical performance of the Immunalysis Opioids panel on the ARCHITECHT c system, which consists of assays that utilize photometric technology for the semiquantitative or qualitative determination of drugs of abuse in human urine.

Methods: Key performance testing including precision, accuracy by recovery, and percent agreement with a commercially available clinical chemistry analyzer were assessed per Clinical Laboratory Standards Institute (CLSI) protocols.

Results: The observed results for precision, accuracy by recovery, and percent agreement of the Immunalysis Opioid Panel on ARCHITECT c8000 are shown in the table below.

Assay	Cutoff (ng/mL)	Precision %CV[a]	Recovery	Percent Agreement (overall)
6-Acetylmorphine (Qual)	10	≤ 0.6	NA	100%
Buprenorphine (Qual)	5	≤ 0.7	NA	100%
Buprenorphine (Semiquant)	5	≤ 3.1	92% - 104%	100%
Oxycodone (Qual)	100	≤ 0.6	NA	100%
Oxycodone (Qual)	300	≤ 1.3	NA	100%
Oxycodone (Semiquant)	100	≤ 2.8	87% - 102%	100%
Oxycodone (Semiquant)	300	≤ 2.6	87% - 102%	100%
Tramadol (Qual)	200	≤ 0.6	NA	100%
Tramadol (Semiquant)	200	≤ 5.3	89% - 98%	100%
EDDP (Qual)	100	≤ 1.3	NA	98%
EDDP (Semiquant)	100	≤ 4.2	93.6% - 114.2%	98%
EDDP (Qual)	300	≤ 1.1	NA	98%
EDDP (Semiquant)	300	≤ 4.4	86.2% - 106.3%	98%
Fentanyl (Qual)	1	≤ 1.6	NA	100%

[a] Precision for qualitative applications was evaluated on absorbance and for semi-quantitative applications on concentration.

Conclusion: The Immunalysis Opioids panel on the ARCHITECT c8000 demonstrated acceptable performance for precision, accuracy by recovery, and percent agreement. These assays are manufactured by Immunalysis and distributed by Abbott for use on the ARCHITECT c systems through the open channel option.

A-062

Evaluation of NOGGIN and ASPORIN as New Biomarkers for the Diagnosis of Nonalcoholic Fatty Liver Disease

G. Hawa[1], T. Jungwirth[1], A. Missbichler[1], S. A. Polyzos[2], J. Kountouras[3]. [1]*FIANOSTICS GmbH, Wiener Neustadt, Austria,* [2]*First Department of Pharmacology, School of Medicine, Aristotle University, Thessaloniki, Greece,* [3]*Second Medical Clinic, School of Medicine, Aristotle University, Thessaloniki, Greece*

Background: NOGGIN is a secreted homodimeric glycoprotein known to inhibit some of the bone morphogenetic proteins (BMPs), which are members of the transforming growth factor-beta (TGF)-β superfamily. ASPORIN is a secreted dimeric extracellular matrix protein, and, like decorin and biglycan, belongs to the der Small Leucine- Rich Proteins (SLRP) class I family. The main biological function of ASPORIN consists in the regulation of TGF-β1 activity to which it binds directly. A pilot study (Polyzos et al.) has recently shown that NOGGIN may be a valuable biomarker for diagnosis of patients with nonalcoholic fatty liver disease (NAFLD). Since the regulatory functions of NOGGIN and ASPORIN do overlap in being both related to the TGF-β superfamily, we speculated that also ASPORIN serum measurements may be implicated in the pathophysiology of NAFLD.

Method: We included 15 patients with simple steatosis (SS), 16 with nonalcoholic steatohepatitis (NASH) and 24 controls without NAFLD into this pilot cross-sectional study. We also determined the influence of vitamin E (400 IU/day) or spironolactone (25 mg/day) plus vitamin E (400 IU/day) for 52 weeks on biomarker serum levels. ASPORIN and NOGGIN levels were measured with a direct fluorescent immunoassay (FIA) based on a recently developed high sensitivity fluorescence enhancement technology (FluoBolt™).

Results: NOGGIN levels have been found to be lower in SS (5.8±1.5 pmol/l) and NASH (8.7±2.4 pmol/l) patients than in controls (13.7±2.7 pmol/l; p for trend=0.040) and to be similarly increased after 2 months of vitamin E or vitamin E/spironolactone therapy. ASPORIN levels were also significantly lower in SS (469±55 pmol/l) and NASH (379±56 pmol/l) patients than in controls (662±37 pmol/l; p-value for trend<0.001), without differing between SS and NASH patients. ASPORIN levels decreased post-treatment in both groups. More specifically, ASPORIN was not different between groups (p=0.14), but decreased within groups over time (p<0.001). There was not significant difference in the group*time interaction (p=0.57).

Conclusion: NOGGIN and ASPORIN may be valuable biomarkers for the diagnosis of NAFLD patients and they may also mediate the favorable effect of vitamin E treatment, although mechanistic studies are needed. Further studies with higher patient numbers are also required to confirm these promising results.

A-063

Evaluation of hsTNT STAT on Roche Cobas e801 Immunoassay Analyzer

S. Lim, M. S. Lim, S. P. Hoo, S. F. Yew, T. C. Aw. *Changi General Hospital, Singapore, Singapore*

Background: Our laboratory has been using the Roche hsTNT STAT 9 minute assay since it was launched in Singapore in 2011 on the Roche Cobas e601 immunoassay analyzer. In June 2018, we upgraded to the cobas e801, the latest Immunoassay Analyzer in the family. This was because it has a higher throughput, smaller footprint, and ability to load supplies without interrupting the sample run as compared to Cobas e601.

Method: We evaluated the analytical performance of hsTNT STAT on the cobas e801 including test imprecision (coefficient of variation - CV%), correlation with the Cobas e602, linearity and limits of quantification. Total Imprecision was assessed using 2 levels of Roche quality control materials daily and an additional level close to the 99th percentile (BioRad) for a period of 6 months (July to Dec 18). The IFCC criteria define an hs-cTn assay, which must have adequate precision (10% CV) at the 99th centile. Correlation studies and limits of quantification were verified using 40 patient samples. Measuring range verifications were done using CAP materials run in duplicates for each level. The analytical speed for hs-TnT was also monitored from the time serum/plasma samples arrive at the analyzer's loading bay (captured by the e601/e801) to availability of results. Statistical analyses were performed using EP evaluator v11.

Results: Total imprecision for Roche level 1 is 2.1% (mean hs-TnT value - 27ng/L) and 1.4% for Roche level2 (mean hs-TnT concentration - 2016ng/L) while the BioRad control was 2.8% and at a mean value hs-TnT of 16ng/L. Correlation and regression analyses showed very close agreement with e602 (r: 0.999, slope: 1.006). At a CV of 10%, the limit of quantitation for hs-TnT on the e801 was < 3.3 ng/L compared to the e601 at 5.22ng/L. The measuring range was verified and is as stated by the manufacturer from 3.0-10,000ng/L. The e601 processed 45.8% of 1254 hs-TnT STAT tests by the 12th minute and 100% at 30 minutes; the e801 processed 32.8% of 1306 hs-TnT STAT tests by the 11th' minute and 100% at 20 minutes.

Conclusion: The hsTNT STAT assay on the cobas e801 demonstrated good analytical performance and exhibits even lower limits of quantitation compared to e601.

A-064

Abbott Alinity c System Sigma Metrics

S. Schneider, V. Petrides, M. Berman, A. Orzechowski. *Abbott, Abbott Park, IL*

Background: A sigma metric is a simple measurement of assay quality comparing the precision and bias performance of an assay to a total allowable error (TEa) goal. This study was conducted to determine sigma metrics on the Alinity c system for 16 clinical chemistry assays from a variety of therapeutic areas. These results supplement the sigma metrics reported on 58 different Alinity c system clinical chemistry assays presented in posters at the 2017 and 2018 AACC meetings. **Methods:** A sigma metric was calculated for each assay and were plotted together on a single method decision chart. The sigma metric was calculated using the equation: sigma = (TEa - bias)/precision, where the 2014 Ricos desirable biologic variability or the 2014 allowable limits of performance from the Royal College of Pathologists of Australasia (RCPA) was used as the TEa goal. To estimate bias, a method comparison study per CLSI EP09-A3 was performed by testing at least 100 samples in duplicate across the analytical measuring interval on the Alinity c and on the Abbott ARCHITECT *c*8000 systems. The bias was determined at a low medically-relevant concentration level using the Passing-Bablok regression model comparing the first replicate of the Alinity c system result to the mean of the ARCHITECT *c*8000 system results. To estimate precision, a precision study was performed per CLSI EP05-A2 by testing controls and panels in replicates of 2 - 3 during 2 runs per day for 20 days. The within-laboratory standard deviation (SD) or percent coefficient of variation (%CV) for the sample with a concentration closest to a low medically-relevant concentration level was used in the sigma metric calculation. **Results:** The method decision chart showed that 56% of the assays demonstrated at least 5 sigma performance at a low, medically-relevant concentration level. All of the assays demonstrated at least 3 sigma performance. **Conclusion:** A majority of the of Alinity c clinical chemistry assays demonstrated at least 5 sigma performance, and all of the assays had at least 3 sigma performance, consistent with the sigma performance observed on 58 previously studied Alinity c clinical chemistry assays. Sigma metrics can be a useful tool for laboratorians to use to compare and monitor assay performance to ensure high quality healthcare for patients.

A-065

Abbott Alinity i System Sigma Metrics

S. Schneider[1], V. Petrides[1], J. Herzogenrath[2], A. Orzechowski[1]. [1]*Abbott, Abbott Park, IL,* [2]*Abbott, Abbott Park, Germany*

Background: A sigma metric is a simple measurement of assay quality comparing the precision and bias performance of an assay to a total allowable error (TEa) goal. This study was conducted to determine sigma metrics on the Alinity i system for 10 immunoassays from a variety of therapeutic areas - cardiac, metabolic, fertility, cancer, and thyroid. These results supplement the sigma metrics reported on 26 different Alinity i system immunoassays presented in posters at the 2017 and 2018 AACC meetings. **Methods:** A sigma metric was calculated for each assay and were plotted together on a single method decision chart. The sigma metric was calculated using the equation: sigma = (TEa - bias)/precision, where the 2014 Ricos desirable biologic variability or the 2014 allowable limits of performance from the Royal College of Pathologists of Australasia (RCPA) was used as the TEa goal. To estimate bias, a method comparison study per CLSI EP09-A3 was performed by testing at least 100 serum or plasma samples in duplicate across the analytical measuring interval on the Alinity i and on the Abbott ARCHITECT *i* 2000_{SR} systems. The bias was determined at a low medically-relevant concentration level using the Passing-Bablok regression model comparing the first replicate of the Alinity i system result to the mean of the ARCHITECT *i* 2000_{SR} system results. To estimate precision, a precision study was performed per CLSI EP05-A2 by testing controls and panels in replicates of 2 - 3 during 2 runs per day for 20 days. The within-laboratory standard deviation (SD) or percent coefficient of variation (%CV) for the sample with a concentration closest to a low medically-relevant concentration level was used in the sigma metric calculation. **Results:** The method decision chart showed that 60% of the assays demonstrated at

least 5 sigma performance at a low, medically-relevant concentration level. All of the assays demonstrated at least 3 sigma performance. **Conclusion:** The majority of Alinity i immunoassays demonstrated at least 5 sigma performance, and all of the assays had at least 3 sigma performance, consistent with the sigma performance observed on 26 previously studied Alinity i immunoassays. Sigma metrics can be a useful tool for laboratorians to use to compare and monitor assay performance to ensure high quality healthcare for patients.

A-066

Anti-Nuclear Antibodies (ANA) Prevalence in Community-Based Patients in Ontario

H. Li[1], W. Hui[1], H. Tran[1], J. Dhaliwal[1], D. Bailey[2], A. Ptolemy[2], J. Healey[1], P. Catomeris[1]. *[1]Dynacare, Brampton, ON, Canada, [2]Dynacare, London, ON, Canada*

Background: Autoantibodies are antibodies that mistakenly target and react with a person's own tissues or organs, causing inflammation, damage, and/or dysfunction of organs or systems, leading to signs and symptoms of autoimmune disorders. Antinuclear antibody (ANA) is the most common type of autoantibody; ANA test is one of the primary tests used in the diagnosis of a suspected autoimmune disorder or to rule out other conditions with similar signs and symptoms.

Objective: Assess the ANA positivity and prevalence patterns in the patient population tested by our regional reference laboratory.

Methods: A total of 99086 patients (67633 females, 31453 males) from 1 to 103 years of age had physician orders for ANA testing over the period of January 2016 to June 2017. These samples were tested using indirect immunofluorescence assay (IFA). Slides were processed (diluted, aspirated and dispensed) on Bio-Rad PhD System and read manually under a fluorescent microscope. A 1:80 dilution was used for initial screening; samples screened positive were sent for further titration.

Results: Male patients had a higher mean/median age (51/53) than females (50/50). ANA was positive in 14.6% of all patients, with a higher prevalence in females (17.5%) than in males (8.3%). A trend of increasing positivity rate in older patients was observed, in both females and males. Altogether 13 patterns were observed, including, from the most prevalent to the least, speckled (40.3%), homogenous (35.5%), speckled+homogenous (11.2%), centromere (5.5%), nucleolar (5.1%), speckled+nucleolar (1.4%), homogenous+nucleolar (0.69%), proliferating cell nuclear antigen (PCNA) (0.21%), speckled discrete (0.03%), rim (0.014%), centromere+ homogenous (0.007%), speckled+golgi (0.007%), speckled+ centromere (0.007%). The most prevalent antibody titer in positive samples was 1:80 (31.7%), followed by 1:160 (30.3%), 1:640 (19.2%), 1:320 (17.9%), and >1:640 (0.9%) Similar distribution of titer was observed between the three major ANA patterns (speckled, homogenous, and nucleolar). The centromere pattern showed a unique distribution, with the most prevalent titer at 1:640, followed by 1:320, 1:160 and 1:80. Centromere Abs-positive patients had the highest age mean/median (61/61), compared to speckled (51/52), homogenous (53/54), and nucleolar (56/56) patterns.

Conclusion: Data from our lab showed that ANA prevalence was higher in women and older individuals. Speckled and homogenous were the most prevalent patterns of ANA. The mean/median of age of ANA-positive patients was significantly different between ANA patterns. 1:80 was the most prevalent antibody titer. A unique distribution of antibody titer was found in centromere-positive patients. This population-based information may assist physicians to make differential diagnosis of autoimmune diseases.

A-067

Extractable Nuclear Antigen Antibodies (ENA Abs) Prevalence in Community-Based Patients in Ontario

H. Li[1], W. Hui[1], J. Dhaliwal[1], H. Tran[1], A. Ptolemy[2], D. Bailey[2], J. Healey[1], P. Catomeris[1]. *[1]Dynacare, Brampton, ON, Canada, [2]Dynacare, London, ON, Canada*

Background: Autoantibodies are antibodies that mistakenly target and react with a person's own tissues or organs, causing inflammation, damage, and/or dysfunction of organs or systems, leading to signs and symptoms of autoimmune disorders. Antinuclear antibody (ANA) is one of the primary tests used in the diagnosis of a suspected autoimmune disorder or to rule out other conditions with similar signs and symptoms. Antibodies against extractable nuclear antigens (ENA Abs) are typically ordered as a follow-up test in patients with positive ANA, to aid in the diagnosis of particular autoimmune disorders.

Objective: Assess the prevalence of ENA Abs in the patient population tested by our regional reference laboratory.

Methods: A total of 25893 patients (18658 females; 7235 males) from 1 to 100 years of age had physician orders for ENA Abs over the period of January 2016 to June 2017. These patients were not exclusively positive of ANA. They may not have had ANA tested or may have had a negative ANA. ENA Abs were screened using an indirect solid phase enzyme immunoassay using a mixture of ENA Abs against SS-A (Ro), SS-B (La), Sm, RNP/Sm, Scl 70 and Jo-1. Samples screened positive had reflexive testing for specific ENA Abs.

Results: Male patients had a higher mean/median age (55/56) than females (52/52). ENA Abs were screened positive in 12.8% of all patients. The positivity rate of females (15.1%) was about 2.3 times of males (6.6%). There was no obvious correlation between positivity rate and patient age in both females and males. A total of 3291 samples screened positive for ENA Abs were further tested for specific ENA Abs. Of these, 81.1% were confirmed positive for at least one ENA Ab, 9.3% were found borderline for at least one ENA Ab and 9.6% were negative for all ENA Abs. SS-A (Ro) Ab had the highest positivity rate (63.6%), followed by SS-B (La) (19.0%), RNP/Sm (17.6%), Sm (5.8%), Scl-70 (2.9%) and Jo-1 (1.5%). No significant correlation was observed between Ab concentration and patient age for SS-A (Ro), SS-B (La), Scl 70 and Jo-1 Abs. However, concentration of RNP/Sm and Sm Abs had an apparent negative correlation with age. A total of 447 samples screened negative were also tested for specific ENA Abs and all of these were negative for specific ENA Abs.

Conclusion: Data from our laboratory showed that ENA Abs prevalence was higher in females, but there was no obvious correlation between positivity rate and patient age. The concordance data showed that the ENA Ab screen test was less specific than the specific ENA Ab test. This population-based information may assist physicians to make differential diagnosis of autoimmune diseases.

A-068

Increased Sensitivity for PR3-ANCA Using a Novel Chemiluminescence Immunoassay

E. Hoxha[1], R. Stahl[1], T. Witte[2], F. Seebeck[3], O. Sendscheid[4], W. Schlumberger[3], C. Daehnrich[5]. *[1]University Hospital Hamburg-Eppendorf, Center for Internal Medicine, Medical Clinic and Polyclinic, Hamburg, Germany, [2]Hannover Medical School, Clinic of Immunology and Rheumatology, Hannover, Germany, [3]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany, [4]EUROIMMUN US, Inc., Mountain Lakes, NJ, [5]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Lübeck, Germany*

Background: ANCA-associated vasculitides (AAV) encompass granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA). In GPA, ANCA are mainly directed against proteinase 3 (PR3), while anti-myeloperoxidase (MPO) antibodies are primarily associated with MPA and EGPA. Sensitive and accurate PR3- and MPO-ANCA detection aids early diagnosis and might enable therapy monitoring in patients with AAV. Due to limitations in sensitivity, narrow measuring ranges and missing quantification of available assays, ANCA are questioned as biomarkers for monitoring disease activity. Novel immunoassays might reduce these limitations and add value to ANCA monitoring. Chemiluminescence immunoassays (ChLIA) and bead technology have lately become an interesting option for a fast, sensitive and accurate detection of PR3-and MPO-ANCA. Here, we investigated the diagnostic performance of the first commercial ChLIA that uses human recombinant PR3 as antigen.

Methods: Sera from 220 clinically characterized AAV patients (101 GPA, 104 MPA, 4 EGPA) and 112 disease controls were retrospectively analyzed with the Anti-PR3 ChLIA based on human recombinant PR3 coated to magnetic beads. The ChLIA was processed on the RA Analyzer 10 (Euroimmun), which provides a shelf life of 84 days for reagents. Samples were tested in parallel with the established Anti-PR3-hn-hr ELISA and Anti-MPO ELISA (Euroimmun).

Results: Using the ChLIA system, the measuring range for PR3-ANCA is broader. This can facilitate the exact monitoring of the PR3-ANCA level in the disease course. The Anti-PR3 ChLIA showed a higher sensitivity for PR3-ANCA in AAV samples than the Anti-PR3-hn-hr ELISA, at a slightly increased specificity (99% vs. 97%). Using ChLIA, the sensitivity for PR3-ANCA rose from 86% to 90% in GPA and from 0% to 6 % in MPA. These results represent the situation for PR3- and MPO-ANCA in the current literature. Although PR3-ANCA have the highest prevalence in GPA, they are also described in MPA. The inter-assay concordance rates were 95% (GPA) and 98% (other AAV, disease controls). Good correlation between different monospecifc assays is important for harmonization of diagnostic testing.

Conclusion: The novel anti-PR3 ChLIA outperformed the ELISA in sensitivity and specificity, thus providing a valuable tool for AAV diagnosis and ANCA target antigen differentiation. Nevertheless, the clinical picture must not be ignored as it influences the therapy. Future studies with larger cohorts and samples from AAV patients under treatment are necessary to further analyze the performance of the novel ChLIA and to study the relevance of PR3-ANCA level monitoring in follow-ups.

A-069

Deep Artificial Neural Networks for Pattern Recognition in Indirect Immunofluorescence Microscopy

S. Gerlach[1], M. Danckwardt[2], C. Marzahl[2], S. Lederer[2], O. Sendscheid[3], J. Mielke[2], K. Fechner[2], W. Stoecker[2], J. Voigt[2]. *[1]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Lübeck, Germany, [2]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany, [3]EUROIMMUN US, Inc., Mountain Lakes, NJ*

Background: Deep artificial neural networks are the backbone of most current state-of-the-art pattern recognition applications. Competitions and benchmarks on public datasets like ImageNet [1], CIFAR [2] or MNIST [3] point up the high performance of deep neural networks in pattern recognition and image classification.

Methods: We trained a deep convolutional neural network for recognizing several nuclear and cytoplasmic indirect immunofluorescence patterns exposed on NMDA-R-transfected HEK293 cells. Our dataset contained 132,000 image patches, of which 33,000 were used for evaluation. The network consisted of convolutional layers for feature extraction and used dropout and batch normalization [4] for regularization. As intermediate activation functions the Rectified Linear Unit was applied. For optimization, Adam [5] was used, which computes adaptive learning rates for each parameter within the network. We applied different data augmentation techniques to generalize on mixed patterns and to obtain more training data. To visualize what features the trained neural network is capable to extract, we generated artificial images that stimulate different layers of neurons. In early layers, edges and lines were detected. Whereas, in higher layers, richer patterns and detailed structures were identified.

Results: Compared to visual result interpretation, the deep neural network achieved 97.7% sensitive recognition of NMDA-R-specific patterns at a specificity of 99.5%. Sensitivities for nuclear patterns ranged from 94.5% to 99.0%, with specificities from 99.2% to 99.9%. Cytoplasmic pattern sensitivities ranged from 96.1% to 98.2% and specificities from 97.0% to 99.3%.

Conclusion: Pattern recognition and image classification with deep neural networks offer a high sensitivity and specificity for images of indirect immunofluorescence microscopy modality. Future releases of the EUROPattern-Suite will support deep neural networks for diagnostics on anti-nuclear antibodies (ANA), antibodies against neutrophil granulocytes (ANCA), cell-based assays and Crithidia luciliae (CLIFT) diagnostics.

1] Russakovsky et al. ImageNet large scale visual recognition challenge. Int J Comp Vision 115(3): 211-252 (2015).

[2] Krizhevsky. Learning multiple layers of features from tiny images (2009).

[3] LeChun et al. The MNIST database of handwritten digits (1998). http://yann.lecun.com/exdb/mnist/

[4] Ioffe and Szegedy. Batch normalization: accelerating deep network training by reducing internal covariate shift. arXiv:1502.03167 (2015).

[5] Kingma and Ba. Adam: a method for stochastic optimization. arXiv:1412.6980 (2014).

A-070

Co-occurrence of Autoantibodies to DFS70 and Autoantibodies Associated with Rheumatic Diseases

B. Teegen[1], W. Meyer[1], O. Sendscheid[2], J. Fraune[1], T. Scheper[1], W. Schlumberger[1], W. Stoecker[1], K. Rentzsch[1]. *[1]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany, [2]EUROIMMUN US, Inc., Mountain Lakes, NJ*

Background: Autoantibodies to DFS70 (dense fine speckles) belong to the group of anti-nuclear antibodies (ANA) and can be found in the context of different diseases, including systemic autoimmune rheumatism, but are also frequently found in healthy blood donors. To assess their diagnostic relevance in rheumatic diseases, we investigated the prevalence of anti-DFS70 and co-existing ANA in a cohort of consecutive patients with suspected rheumatic diseases.

Methods: 1022 consecutive samples, sent to a reference laboratory (Lübeck, Germany) for determination of antibodies against DFS70 or ANA differentiation, were incubated using a special immunoblot which combines the antigens nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl100, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-protein, AMA-M2 and DFS70 (EUROLINE ANA Profile 3 plus DFS70, Euroimmun, Germany).

Results: Anti-DFS70 were detected in 326 (31.9%) patient samples. 239 (73.3%) of these reacted only with DFS70, the other 87 samples (26.7%) displayed anti-DFS70 plus one or more other ANA specificities.

Patients positive for	n	Anti-DFS70 pos. (%)
AMA-M2	39	20.5
Rib. P-protein	26	19.2
Histones	15	40.0
Nucleosomes	44	25.0
dsDNA	98	28.6
PCNA	22	31.8
CENP-B	55	30.9
Jo-1	17	11.8
PM-Scl	14	28.6
Scl-70	29	17.2
SS-B	43	9.3
Ro-52	143	18.2
SS-A	105	15.2
Sm	9	11.1
RNP/Sm	32	6.3

Conclusion: A great number of positive ANA are caused by anti-DFS70, which do not show any disease specificity. Testing of anti-DFS70 may provide an explanation for positive ANA patterns in indirect immunofluorescence on HEp-2 cells to which no distinct, disease-specific ANA can be attributed. The presence of anti-DFS70, however, does not replace diligent ANA differentiation.

A-071

Laboratory Diagnostics of Autoantibodies in Autoimmune Myopathies

O. Sendscheid[1], W. Meyer[2], T. Scheper[2]. *[1]EUROIMMUN US, Inc., Mountain Lakes, NJ, [2]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany*

Background: Idiopathic inflammatory myopathies (IIM) are rare autoimmune diseases of skeletal muscle, potentially associated with underlying tumors. The main forms are polymyositis (PM), dermatomyositis (DM), necrotizing myopathy (NM) and sporadic inclusion body myositis (sIBM). IIM are characterized by a diverse range of autoantibodies. Their specificities provide an indication of the disease subform. The combined frequency of all autoantibodies in myositis amounts to around 60%, whereby there is virtually no overlap in the occurrence of the different antibodies. Therefore, comprehensive multiparametric testing is essential to maximize the diagnostic information obtained from the analysis.

Methods: In suspected cases of PM/DM, patient sera are serologically investigated using an indirect immunofluorescence assay (IFA) on a substrate combination of HEp-2 cells and primate liver, with confirmation of results by monospecific tests. Since antibodies against the cytoplasmic antigens are sometimes not clearly detectable with IFA, parallel performance of the screening and confirmatory test is recommended. Multiparametric immunoblots are an ideal confirmatory method, as they enable monospecific and simultaneous detection of many different antibodies.

Results: A total of 804 sera from myositis patients and 786 control sera were investigated using a line immunoblot containing 16 antigens to detect the most relevant myositis-specific and -associated auto-antibodies. The antibody prevalences obtained in the myositis patients ranged from 1% (anti-EJ, -OJ) to 21% (anti-Jo-1), while the specificities for the individual antigens amounted to 97-100%. In another study, a novel Anti-cN-1A ELISA provided a diagnostic sensitivity for sIBM of 35-39% at a specificity of 96% (Table).

Autoantibodies against	Prevalence	Specificity
Mi-2α	7 %	100 %
Mi-2β	3-4 %	≥98 %
TIF1γ	-	100 %
MDA5	2 %	100 %
NXP2	2 %	100 %
SAE1	4 %	100 %
Ku	3-5 %	≥95 %
PM-Scl100	4-7 %	100 %
PM-Scl75	6 %	98 %
Jo-1	12-21 %	100 %
SRP	4 %	99 %
PL-7/PL-12	2-4 %	100 %
EJ	1 %	100 %
OJ	1 %	100 %
HMGCR	6 %	-
cN-1A	35-39 %	96 %

Conclusion: The determination of myositis-specific and myositis-associated autoantibodies can significantly reduce the time to diagnosis, with multiparametric testing ensuring the highest serological detection rate. Immunoblots are ideal for multiplex confirmatory testing as they offer broad antigen combinations, easy interpretation and full automatability. It is anticipated that ongoing research will identify further novel autoantibodies in myositis.

A-072

Improvement in Clinical Outcomes When Handling Critical Patients using NephroCheck® Test - A Pilot Study in Chile

J. Garate[1], S. Cuéllar[2], M. Luque[1], M. Jerez[2], K. Vergara[1], J. Aldunate[2]. [1]*Clínica Redsalud Vitacura, Santiago, Chile,* [2]*Laboratorio Clínica Redsalud Vitacura, Santiago, Chile*

Background: The diagnosis of acute kidney injury (AKI) is based on urine output and GFR, two markers of kidney function. This definition has been challenged in recent years, since the usage of two novel urine markers: TIMP-2 & IGBP-7, described to correlate with kidney injury progression, even before serum creatinine (sCr) is affected. These markers are available for IVD under the name of NephroCheck® (NC) test. Nonetheless, there's still a gap in clinical adoption and interpretation of these markers as well as a lab opportunity to deliver results when needed. Currently, there's no evidence of clinical usage of NC in Latin America. In Chile we started a patient study to develop local experience for proper clinical usage of this test. **Objective:** Determine the clinical utility of the NephroCheck® test in Chilean population to assess and help to prevent AKI in critical patients and standardize clinical behaviors adopted in ICU based upon results. **Methods:** We developed a testing profile for every admission in ICU, including the urine NC test and sCr both run in VITROS® 5600 systems from Ortho Clinical Diagnostics Inc. NC risk scores of developing AKI in next 12h are: ≤0.3 low risk, 0.3 - ≤2.0 moderate risk and >2.0 high risk. Two groups were defined, according to NC scores: Group A >0.3 and Group B ≤0.3. Kidney failure was diagnosed based on KDIGO criteria. Patient clinical data was retrieved from HIS. Patients having Chronic Kidney Disease at any stage, more than 85 years, >100 kg or <55 kg, liver disease with a Child-Pugh score Class B or 1.5 times above normal limit of transaminases, pregnancy and lactation and/or under treatment using COMT or MAO inhibitors were excluded. Both groups were treated with the same nephroprotective behaviors from AKI bundle, driven by NC results. Data analysis was performed using SPSS v 13.0. **Results:** A total of 26 patients were included in the study (55.2 ± 18.3, male 46.2%). The distribution per admissions were: septic shock 34.6%, post-surgery 19.2% and nephrotoxic intake 33.6%. No significant differences were observed in age distribution between groups (A:54.1 ± 14.1 vs B:55.9 ± 20.7; p=0.8), neither in average basal sCr values at admission in ICU: (A:0.86±0.53 v/s B:0.71±0.30 mg/dl; p=0.36). SOFA (Sequential Organ Failure Assessment) score revealed group A contained more severe patients (A:5.45 vs B:2.73; p= 0.02). In group A, 27.3% of the patients (3) developed AKI. Only 9.1% developed AKI stage 2/3.Only 6.7% evolved to AKI stage I in group B. **Conclusions:** The adoption of NC in ICU plays a key role in patient's outcome while in ICU stay, since none of studied cases required Renal Replacement Therapy (RRT), neither dialysis nor High volume hemofiltration (HVH). Nephroprotective behaviors were adopted at an earlier stage, compared to sCr guided decisions, reducing the incidence of AKI from 40% to 27,3% in our ICU, according to historical records.

A-073

Expression and Significance of Caspase-1 in Patients with Systemic Lupus Erythematosus

Q. Dong, K. Chen, S. Cao, W. Wang. *Zhongshan Hospital of Sun Yat-sen University, ZhongShan, China*

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease involving multiple system, organs, and autoantibodies. Immune dysfunction plays an important role in the formation and development of SLE, especially, the recent found immune regulation mechanism, inflammasome, which has been involved in many immune diseases. The aim of the study was to investigate the expression and clinical significance of caspase-1, the central enzyme of the inflammasome signaling pathway, in the patients with systemic lupus erythematosus (SLE).

Methods: The current study was conducted at the Zhongshan hospital of Sun Yat-sen University laboratory. 74 patients with SLE and 20 healthy control were included in the study. Patients with SLE in accordance with the American College of Rheumatology classification criteria (ACR 2009) for the dignosis of SLE were included in the study, while, patients with liver or kidney disease, infection, cancer, metabolic diseases, and other autoimmune diseases were excluded. SLE disease activity index (SLEDAI) of each patients was determined by clinical and serological data including skin involvement, arthritis, renal involvement, full cell blood count, serum complement levels, anti-dsDNA and other extractable nuclear autoantibodie.ELISA was used to detect the serum levels of caspase-1 in patients and health controls. Correlations between caspase-1 expression and clinical or laboratory parameters were analyzed by SPSS statistical tool.The Mann-Whitney test was used to comparisons between quantitative variables and the Spearman correlation analysis to study the correlation between variables.

Results: The study subjects had a mean age of 33.50±11.48 years (SLE) and 36.72±13.43 years (controls).Compared to those in healthy controls, levels of caspase-1 was lower in SLE patients (55.96±30.130 *pg*/mL vs.74.61±20.384 *pg*/mL, p<0.05); the levels of caspase-1 was inversely correlated with SLEDIA scores (r=-0.313, p=0.006), globulin (r=-0.268, p=0.024) and Anti-dsDNA (r=-0.422, p=0.001), whereas positively correlated with TC (r=0.326, p=0.024) and C3 (r=0.352, p=0.003). Further, our study showed that the patients with disease in remission (SLEDAI=0) exhibited higher serum levels of caspase-1 (69.64±30.354 *pg*/mL vs.50.24±26.63 *pg*/mL, p<0.05) .

Conclusions: Our data indicated that the expression of caspase-1was inversely correlated with the disease activity, suggesting it might play a protective role in the pathogenesis of SLE.

A-074

A Simple Method for Lymphocyte-Subset Dynamic Analysis

S. So[1], D. K. So[1], M. Irago[1], K. Ukita[1], S. Makino[1], H. Kuroda[2], T. Yonedo[3], T. Chen[4]. [1]*Non-invasive Cancer Therapy Research institute (NiCTRi), Tokyo, Japan,* [2]*Sekiminato Memorial Medical Corporation Green Clinic, Tochigi, Japan,* [3]*Department of General Medicine, Hijirigaoka Hospital, Tokyo, Japan,* [4]*Non-invasive Cancer Therapy Research institute Taiwan (NiCTRi-Tw), Taipei, Taiwan*

Background: Despite medical advancement in clinical and diagnostic immunology, clinical laboratory data of white blood cells (WBC) in complete blood count (CBC) is still limited to the 5 subpopulations of granulocytes (neutrophil, eosinophil, basophil), monocytes, and lymphocytes. A patient's lab result, especially the primitive response lymphocyte subsets such as Natural Killer cells (NK) and NKT cells is critical in understanding the direction of the patient's immunity. This study proposes a simple, cost-friendly method to further understand the immunity's dynamic changes which adds to the current methodology by also monitoring additional 5 lymphocytes subsets of NK, NKT, Helper-T, Killer-T, and B cell. Monitoring beyond the 5 subpopulations of granulocytes (neutrophil, eosinophil, basophil), monocytes, and lymphocytes, our proposed method can provide us with more detailed data that can be used to interpret our immune system for clinical diagnosis and prognosis.

Methodology: Using two color flow cytometry, we identified and differentiated lymphocyte count and percentage of Cluster of Differentiation (CD) markers in different clinical settings. Of the full array of patients seen over the years, this study focused on the distribution of the lymphocytes and its CD markers were identified in a) healthy individuals, b) cancer patients undergoing chemotherapy, c) untreated progressive

cancer patients after injecting back the amplified autologous NK cell, and d) patients with influenza displaying acute viral infections. The specific sample of patients were selected, for the purposes of this presentation, to portray the variety of patients, not limited to cancer patients, that benefit from this method of dynamic analysis.

Results: In healthy individuals, the immune system shifted to Th1 cellular immunity, showing increased Killer-T cells *CD8* subset after one round of NK cell infusion ($5.5*10^8$ NK cell, purity 92%). When accompanied with an increased NK cell infusion, there was a trend of slow shift to ThP, yielding increased Helper-T cells *CD4* subset. In cancer patients undergoing chemotherapy with increased NK cell infusions, Helper-T cells *CD4* and Killer-T cells *CD8* subsets both showed an increase in numbers at the same time. With one untreated progressive breast cancer patient undergoing only NK cell immunotherapy, morphologic improvement on images were observed with overall immune enhancement similar with the patients undergoing chemotherapy. With influenza patients that display acute viral infections, the early stage in infection showed a remarkable decrease in Helper-T cells CD4 subset despite NK cell subset persistence. However, NK cell subset decrease as Helper-T cell CD4 subset increase during the recovery stage. This result demonstrates the importance of innate immunity in immunoevaluation.

Conclusion: Using our lymphocyte analysis method, these dynamic changes of our immunity were noted as follows:

1. Helper T-cell CD4 subset as an essential center in adaptive immunity

2. NK cell plays a booster role in Th1 cellular immunity and maintains the Helper T-cell CD4 subset's number

3. A self-regulated immune-compensation may exist

A-076

Biochemical and Anthropometrical Parameters for Pregnant Women in General Hospital Dr. Raymundo Abarca Alarcón, from Guerrero, Mexico

N. R. E. Barlandas Rendon[1], S. Quintana Ponce[1], O. Y. Nuñez Ramos[1], M. Ramos García[2], M. Valle de la Paz[1]. [1]*Autonomous University of Guerrero, Chilpancingo, Mexico,* [2]*Health Secretary of Guerrero, Chilpancingo, Mexico*

Gestational diabetes (GD) is defined as the carbohydrate intolerance diagnosed in pregnancy, usually starting at 20 weeks of gestation, a condition called insulin resistance, risk factors such as age, obesity, family history of diabetes, GD in previous pregnancies, and abortions can have an important influence on the development of GD. The objective of this research is to evaluate the biochemical and anthropometric parameters and the development of GD in pregnant women who come to the General Hospital Dr. Raymundo Abarca Alarcón, Chilpancingo, Guerrero, Mexico.

Materials and methods: This transversal descriptive research was made since July 2016 and finished in June 2017 in General Hospital Dr. Raymundo Abarca Alarcón, Chilpancingo, Mexico. 120 patients were analyzed with more than 20 weeks of gestation. Those patients were given talks about gestational diabetes. They were asked to sign consent. Later, they answered some risk factors questions, anthropometric measurements were obtained, arterial pressure measurements, urine and fasting blood were collected. They took 50 g of glucose and one hour later, a second blood sample was taking for performing the blood glucose test to screen for gestational diabetes.

Results: 54.6% of patients answered to have relatives with diabetes, 16.06% of mothers' patients had diabetes. 13.22% said their father had diabetes and 8.28% of women had microalbuminuria. The mean Body Mass Index (BMI) (kg/m^2) was 27. According to the World Health Organization, that is overweight.

Conclusion: 3.3% of patients meet the criteria to be diagnosed with gestational diabetes, 25.8% of patients turned out to be glucose intolerant and 70.8% of them were normal.

A-077

Insulin Resistance is a Risk Factor in the Progression of Hepatocellular Carcinomapatients after Hepatectomy

Y. Yao, D. Yang, Y. Huang, Y. Chen. *First Affiliated Hospital, College of Medicine, Zhejiang University, HangZhou, China*

Background: To explore whether baseline homeostasis model assessment of IR (HOMA-IR) were associated with the progression in patients with HCC who underwent liver resection.

Methods: From 1 January 2016 to 2 July 2016, a total of 144 HCC patients admitted to our hospital were enrolled, 105 ages and gender matched healthy control subjects were also enrolled. HOMA-IR and other tumor-related indexes were collected. 144 HCC patients were divided into two groups and analyzed the differences according to the median level of HOMA-IR. The HOMA-IR values were also checked between recurrence and non-recurrence patients. Spearman's correlation test was used to find correlation between HOMA-IR and other clinic laboratory indexes. Cox's proportional hazards regression mode was used to determine potential risk factors for ER. Kaplan-Meier analysis (log-rank test) was performed to compare the cumulative incidence of recurrence between independent risk factor's subgroups.

Results: HOMA-IR ($Z = 3.82$, $P < 0.001$), Aspartate aminotransferase (AST) ($Z = 6.23$, $P < 0.001$), Alanine transaminase (ALT) ($Z = 5.84$, $P < 0.001$) and blood platelet (PLT) ($Z = 7.21$, $P < 0.001$) in 144 HCC patients were significantly increased compared with healthy controls. The significant differences of BMI ($Z=2.18$, $P = 0.046$), BCLC score system ($Z = 3.35$, $P = 0.001$) and recurrence events ($Z = 2.36$, $P = 0.018$) were also noticed between high and low HOMA-IR groups. HOMA-IR levels in BCLC 0 to C were steady increased ($\chi^2 = 32.79$, $P < 0.001$). Moreover, patients with cancer recurrence have higher levels of HOMA-IR ($Z = -2.47$, $P = 0.014$) than patients without. HOMA-IR ($r = 0.610$, $P < 0.001$), fasting insulin ($r = 0.503$, $P < 0.001$) and fasting c peptide ($r = 0.599$, $P < 0.001$) were correlated with BMI. HOMA-IR ($r = 0.363$, $P= 0.021$) and fasting c peptide ($r = 0.524$, $P<0.001$) were also correlated with serum triglyceride (TG).Univariate and multivariate analysis indicated that High BCLC stage ($OR = 2.479$, $P = 0.003$) and HOMA-IR ($OR = 2.336$, $P = 0.005$) were two independent risk factors for recurrence. The presence of higer HOMA-IR (47.3% vs. 23.9%, P = 0.002) and BCLC stage (56.3% vs. 19.2% P < 0.001) were significantly associated with higher cumulative incidence of early recurrence.

Conclusion: A high preoperative HOMA-IR was significantly associated with high BCLC stage and increased risk of recurrence in HCC patient after liver resection.

A-078

A Comparison Study on the Test Results of Serum Tumor Marks Conducted by Four Different Automatic Analysis Systems

J. Zhang. *Laboratory medicine, HangZhou, China*

Background: The method of serum tumor marks testing by automatic analysis system has significant values in the areas of early screening, treatment assessment, prognosis, recurrence monitoring. The purpose of this paper is to get an understanding of four automatic analysis systems, namely, Architect i2000 (Abbott Diagnostics, Abbott Park, IL),AxSYM (Abbott Diagnostics),Advia Centaur (Siemens Diagnostics, Tarrytown, NY) and E170(Roche Diagnostics, Indianapolis, IN) to pinpoint the consistencies of test results amongst seven tumor marks AFP,CEA,CA 125,CA 19-9,Ferritin,total PSA(tPSA) and freee PSA(fPSA).

Methods: To conduct imprecision analysis on AFP,CEA,CA 125,CA 19-9,Ferritin,tPSA and fPSA of four automatic analysis systems in terms of imprecision, and, to analyze on the consistence of the test results of the above-mentioned seven tumor marks.

Results: The results shows that the imprecison of AFP,CEA,CA 125,CA 19-9,Ferritin,tPSA and fPSA is in the range of 2.8% and 6.5% by the four automatic analysis systems. The r coefficient on the test results of AFP,CEA,CA 125,CA 19-9,Ferritin,tPSA and fPSA by the four automatic analysis systems is 0.996,0.987,0.993,0.804,0.994,0.993 and 0.988(AxSYM and Architect i2000);0.995,0.965,0.990,0.975,0.991,0.993 and 0.953(Advia Centaur and Architect i2000);0.993,0.985,0.990,0.815,0.989,0.990 and 0.972(E170 and Architect i2000)respectively; the slope of them is 0.962,0.846,0.849,0.399,0.852,0.976 and 1.086(AxSYM and Architect i2000);0.922,0.609,0.846,0.625,0.874,0.931 and 0.826(Advia Centaur and Architect i2000);1.014,1.227,0.999,0.620,1.102,0.945 and 0.884(E170 and Architect i2000)respectively.

Conclusion: The test results of AFP,CA 125,Ferritin and tPSA by four anlalysis systems is highly identical whilst the results between CEA,CA 19-9 and fPSA differs to some extent, especially in the case of CA 19-9 which means the standardisation of the tumor mark testing is to be further improved.

A-079

A Comparison Study on the Test Results of Serum Tumor Marks Conducted by Four Different Automatic Analysis Systems

J. Zhang. *Laboratory medicine, HangZhou, China*

Background: The method of serum tumor marks testing by automatic analysis system has significant values in the areas of early screening, treatment assessment, prognosis, recurrence monitoring. The purpose of this paper is to get an understanding of four automatic analysis systems, namely, Architect i2000 (Abbott Diagnostics, Abbott Park,

IL),AxSYM (Abbott Diagnostics),Advia Centaur (Siemens Diagnostics, Tarrytown, NY) and E170(Roche Diagnostics, Indianapolis, IN) to pinpoint the consistencies of test results amongst seven tumor marks AFP,CEA,CA 125,CA 19-9,Ferritin,total PSA(tPSA) and freee PSA(fPSA).

Methods: To conduct imprecision analysis on AFP,CEA,CA 125,CA 19-9,Ferritin,tPSA and fPSA of four automatic analysis systems in terms of imprecision, and, to analyze on the consistence of the test results of the above-mentioned seven tumor marks.

Results: The results shows that the imprecison of AFP,CEA,CA 125,CA 19-9,Ferritin,tPSA and fPSA is in the range of 2.8% and 6.5% by the four automatic analysis systems. The r coefficient on the test results of AFP,CEA,CA 125,CA 19-9,Ferritin,tPSA and fPSA by the four automatic analysis systems is 0.996,0.987,0.993,0.804,0.994,0.993 and 0.988(AxSYM and Architect i2000);0.995,0.965,0.990,0.975,0.991,0.993 and 0.953(Advia Centaur and Architect i2000);0.993,0.985,0.990,0.815,0.989,0.990 and 0.972(E170 and Architect i2000)respectively; the slope of them is 0.962,0.846,0.849,0.399,0.852,0.976 and 1.086(AxSYM and Architect i2000);0.922,0.609,0.846,0.625,0.874,0.931 and 0.826(Advia Centaur and Architect i2000);1.014,1.227,0.999,0.620,1.102,0.945 and 0.884(E170 and Architect i2000)respectively.

Conclusion: The test results of AFP,CA 125,Ferritin and tPSA by four anlalysis systems is highly identical whilst the results between CEA,CA 19-9 and fPSA differs to some extent, especially in the case of CA 19-9 which means the standardisation of the tumor mark testing is to be further improved.

A-080

Novel Monoclonal Anti-Müllerian Hormone Antibodies Applicable to Sensitive Diagnostic Immunoassays

S. Tiitinen, S. Eklin, T. Hämäläinen, M. Mattila, L. Kiiskinen. *Medix Biochemica, Espoo, Finland*

Background: Anti-Müllerian hormone (AMH), is a glycoprotein hormone produced by the Sertoli cells of the testis and by the granulosa cells of the ovarian follicles. AMH has traditionally been used as a biomarker to investigate gonadal development and abnormal sexual differentiation in newborns. More recently, it has also been widely adopted as a biomarker to assess ovarian reserve levels and to predict response to controlled ovarian stimulation in fertility treatments.

AMH is synthesized as a homodimeric precursor (proAMH), which is cleaved to yield an amino-terminal dimer (AMH_N) and a C-terminal dimer (AMH_C). These dimers associate noncovalently to form a tetrameric AMH complex. Both proAMH and the cleavage products can be present in circulation and in target organs. This complexity of AMH protein presents challenges to the development of specific, high-affinity AMH antibodies.

We have developed six novel mouse monoclonal antibodies against human AMH. The binding properties of these antibodies were studied in fluorescence-based immunoassays (FIA) with purified AMH protein, commercial AMH control reagents, and with serum samples containing varying levels of AMH.

Methods: The best antibody pairs for sandwich assays were determined using commercial control reagents (Seronorm Immunoassay Liq L-1, L-2, and L-3), with reported AMH levels of 5.1, 3.7, and 1.6 ng/mL, respectively. Biotin-conjugated antibodies and europium-labeled streptavidin were used to detect the AMH protein captured by each antibody. Standard curves for selected antibody pairs were generated on sandwich FIA, using purified recombinant AMH_N in a buffer solution and native AMH in serum at concentrations ranging from 0.005 to 100 ng/mL. The best pairs were also used to study AMH levels in 48 serum samples with AMH levels varying from 0.03 to 23.8 ng/mL, as measured on Beckman Coulter DxI 800 or on Roche cobas 8000 analyzers.

Results: Out of the six antibodies studied, several well-performing antibody pairs for AMH detection were identified. None of the antibodies worked as a pair with itself, which might have been possible taking into consideration the dimeric nature of AMH.

Standard curves with the best pairs, originating from hybridomas designated as 11302, 11303, or 11306, indicated linear assay performance in the concentration range tested. Serum AMH level results measured with these antibody pairs correlated well with results obtained on commercial diagnostic platforms; the highest correlation coefficient values were above 0.980.

Conclusions: These results demonstrate that the novel monoclonal AMH antibodies developed can bind the biochemically challenging target antigen both as a recombinant protein in buffer solution and as a native protein in serum. These antibodies can be used as tools to develop sensitive diagnostic assays for measuring AMH levels in clinical samples.

A-081

Evaluation of the IgE Assay for Use on the Binding Site Optilite® Analyser

J. Seibaka[1], S. Caspari[2], B. Weber[2], M. Grimmler[2], F. Murphy[1], E. Jenner[1], M. McCusker[1], S. Harding[1], D. Matters[1]. *[1]The Binding Site Group Ltd, Birmingham, United Kingdom, [2]DiaSys Diagnostic Systems GmbH, Holzheim, Germany*

Background: The Optilite Immunoglobulin E (IgE) reagent is intended for the quantitative in vitro measurement of IgE in serum using the Binding Site Optilite analyser. Measurement of IgE can be used as an aid in the diagnosis of abnormal protein metabolism and the body's lack of ability to resist infectious agents. Human IgE has a molecular weight of about 190 000 Dalton and consists of two identical heavy chains and two identical light chains which are bound together by disulphide bonds in a characteristic Y-shaped form. One of the functions of IgE is the specific defense against parasites. In developed countries it plays a major role in the mediation of immediate type hypersensitivity reactions (type I according to Coombs and Gell). Harmless, polyvalent antigens (pollen, house dust mites), stimulate B cells at the site of entry to synthesize specific IgE which in part binds to mast cells. The half-life of plasma IgE is 2-3 days while mast cell-bound IgE has a half-life from months to years. During the next contact of the antigen with the sensitized mast cell, bound IgE are cross linked. The cell is degranulated and mediators (mainly histamine) are released which cause, for example, symptoms of hay fever, asthma, and atopic eczema. Elevated IgE levels occur in atopic diseases, parasitic infection, diseases with T cell dysfunction, certain malignant tumours (respiratory tract, gastrointestinal tract), hyper-IgE syndrome, graft-versus-host disease, and severe burns. Measurement of total IgE is mainly conducted in the field of the diagnosis of atopic diseases where highly increased IgE levels may occur. IgE testing is a good tool especially in differential diagnostic examination of clinical pictures with possible allergic background. Here we describe the performance of an immunoassay for the detection and quantification of IgE on the Binding Site Optilite analyser.

Methods: A linearity study was performed according to CLSI EP06-A. Limit of Quantitation (LoQ) was verified using a study based on CLSI EP17. Correlation to an alternative commercially available assay was completed using 144 clinical samples. A precision study was performed according to CLSI EP5-A2 over a period of 5 days using one reagent lot on one analyser. 4 samples with different analyte concentrations were tested. Interference testing was performed following CLSI EP7-A2 using 5 potential interferents.

Results: The assay was shown to be linear over a range of 3.6 - 1351.5 lU/mL at the standard 1+4 analyser dilution. The (LoQ) for this assay was defined as the bottom of the overall measuring range, 4 IU/mL. Comparison demonstrated good agreement (Passing and Bablok slope y=0.97x+0.59, correlation by linear regression r=0.999). The between run precision coefficients of variation (CVs) were as follows: 5.34% at 84.8 IU/mL, 3.19% at 106.0 IU/mL, 1.66% at 240.3 IU/ml and 2.61% at 754.6 IU/mL. No significant interference effects were observed when testing Intralipid (176 mg/dL), unconjugated bilirubin (68.5 mg/dL), conjugated bilirubin (74.3 mg/dL), Rheumatoid factor (446.5 IU/mL) and haemoglobin (575 IU/mL).

Conclusion: The Optilite IgE assay provides a reliable and precise method for quantifying IgE content in serum and correlates well with existing methods.

A-082

Development of a Duplex Biochip Assay for the Simultaneous Detection of Anti-thyroglobulin and Anti-thyroid Peroxidase Antibodies on the Fully Automated Evidence Evolution Analyser

T. Doherty[1], R. McGinley[1], E. Harte[1], C. Richardson[1], A. M. Jennings[2], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: Autoantibodies (aAb) are established clinical markers of autoimmune disease, aiding in the diagnosis of autoimmune thyroid disease (AITD) and distinguishing it from other forms of thyroiditis. AITD causes cellular damage and alters thyroid gland function by humoral and cell mediated mechanisms. A characteristic feature of AITD is the production of aAb to Thyroglobulin (Tg) and Thyroid Peroxidase (TPO), key regulatory proteins in the synthesis of the hormone thyroxine. Elevated serum levels of TgaAb and TPOaAb have been shown to be associated with chronic thyroiditis such as Hashimotos' Thyroiditis (which results in hypothyroidism) and Grave's disease (which results in hyperthyroidism). TPOaAb are considered a more sensitive marker of thyroid autoimmunity; however, depending on the patient, TPOaAb may be low while TgaAb is elevated, thus dual measurement of TPOaAb and TgaAb will facilitate a more accurate diagnosis of thyroid autoimmunity. Bio-

chip Array Technology (BAT) enables the simultaneous detection of multiple analytes from a single sample. The aim of this study was to develop a duplex biochip based immunoassay, enabling the simultaneous detection of TgaAb and TPOaAb from a single serum sample. By applying these assays to the fully automated, random access Evidence Evolution biochip analyser, the first result can be produced in 37 minutes and one result per minute thereafter, enabling rapid sample analysis. **Methods:** Human TPO and Tg were immobilized to discrete testing regions (DTR) on a biochip surface using a chemiluminescent indirect sandwich assay format and applied to the Evidence Evolution biochip analyser. Assay performance characteristics, including functional sensitivity and precision, were evaluated in accordance with Clinical and Laboratory Standards Institution (CLSI) guidelines. Sample evaluation (n= 100) was carried out with this system and referenced to another commercially available methodology to determine a cut-off value. In addition, a comparative study between the reported duplex approach and another commercial platform was conducted on an independent cohort of samples (n=46) to validate the cut-off and investigate result agreement.

Results: Ten-point calibration curves for each individual analyte were simultaneously generated with assay ranges 0-1500 IU/mL (TgaAb) and 0-1000 IU/mL (TPOaAb). Functional sensitivity was recorded as 5 IU/mL (TgaAb) and 0.75 IU/mL (TPOaAb). Total assay precision, expressed as CV(%), was typically 8.79% (TgaAb) and 10.55% (TPOaAb). The cut-off values were 65.18IU/mL (TgaAb, AUC 0.986) and 34.96IU/mL (TPOaAb, AUC 0.957). The comparative study on an independent cohort of samples showed a 91.3% agreement (TgaAb) and a 100% agreement(TPOaAb).

Conclusion: This study indicates optimal analytical performance of the developed duplex biochip based immunoassay for the rapid and simultaneous measurement of TgaAb and TPOaAb from a single serum sample on the high throughput Evidence Evolution analyser. This application facilitates the clinical investigation on thyroid autoimmunity by providing more information than single analyte testing for the measurements of these analytes.

A-083

Performance Evaluation of Representative Immunoassays from the Comprehensive Metabolic Panel on the Alinity i System from Abbott Laboratories

K. Krishnan[1], C. Rudolph[1], J. Shepard[1], M. Moore[1], J. Herzogenrath[2]. *[1]Abbott Labs, Abbott Park, IL, [2]Abbott Labs, Wiesbaden, Germany*

Background: The Abbott Comprehensive Metabolic Panel/CMP, provides diagnostic information on a variety of analytes that reflect the function of several major organs including the liver, kidney and heart. Abbott metabolic assays are designed to provide laboratory professionals with the clinical information they need to help identify a particular disease state for patients who present with non-specific symptoms. The Alinity i system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved workflow, and greater throughput with up to 200 tests per hour. The Alinity i system has an increased reagent load capacity, holding up to 47 immunoassay reagents, onboard Quality Control (QC), clot and bubble detection ability, and a dedicated pretreatment lane to provide consistent and reliable results.

Objective: To demonstrate the analytical performance of representative assays from the Comprehensive Metabolic Panel of the Alinity i system, which consists of assays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for the quantitative determination of analytes in human serum, plasma, whole blood, or urine.

Methods: Key performance testing including precision, limit of quantitation (LoQ), linearity, and method comparison were evaluated per Clinical and Laboratory Standards Institute (CLSI) guidance. The inputs to the Analytical Measuring Interval include the observed LoQ, imprecision across the range, and the linear range.

Results: The observed results for LoQ, precision, linear range, method comparison to ARCHITECT using Passing-Bablok regression, and determined Analytical Measuring Interval for the representative assays in the Metabolic Panel are shown in the table below.

Assay	LoQ	Total %CV/ SD[a]	Linear Range	Method Comparison (Slope/ Correlation)	Analytical Measuring Interval
Folate	2.2 ng/mL	≤ 8.1/0.29	2.9 to 21.6 ng/mL	0.97/0.96	2.2 to 20.0 ng/mL
C peptide	0.03 ng/mL	≤ 3.3	0.02 to 38.01 ng/mL	0.94/1.00	0.03 to 30.00 ng/mL
Homocysteine	0.79 µmol/L	≤ 6.0	0.43 to 58.38 µmol/L	0.97/1.00	1.00 to 50.00 µmol/L
Insulin	1.6 µU/mL	≤ 2.2	0.2 to 308.4 µU/mL	0.98/1.00	1.6 to 300.0 µU/mL
Anti-CCP	0.4 U/mL	≤ 5.4	0.1 to 195.6 U/mL	0.94/0.99	0.5 to 195.6 U/mL
Intact Parathyroid Routine	0.9 pg/mL	≤ 4.3	0.0 to 3726.2 pg/mL	0.94/1.00	3.0 to 3000.0 pg/mL
Intact Parathyroid STAT	1.0 pg/mL	≤ 5.7	0.0 to 3022.6 pg/mL	0.90/1.00	4.0 to 2500.0 pg/mL
Cortisol	1.0 µg/dL	≤ 5.1	0.4 to 67.5 µg/dL	1.00/1.00	1.0 to 59.8 µg/dL
Ferritin	1.98 ng/mL	≤ 5.5	0.88 to 1675.56 ng/mL	1.01/1.00	1.98 to 1675.56 ng/mL
Pepsinogen-I	0.6 ng/mL	≤ 3.9	0.3 to 264.0 ng/mL	1.01/1.00	0.6 to 200.0 ng/mL
Pepsinogen-II	0.6 ng/mL	≤ 5.3	0.4 to 122.2 ng/mL	0.97/1.00	0.6 to 100.0 ng/mL
25-OH Vitamin D	3.5 ng/mL	≤ 9.0	2.9 to 154.2 ng/mL	1.00/1.00	3.5 to 154.2 ng/mL
Vitamin B12	148 pg/mL	≤ 7.9	129 to 2376 pg/mL	1.04/0.99	148 to 2000 pg/mL
Active B12	1.6 pmol/L	≤ 4.1	4.1 to 134.5 pmol/L	0.95/1.00	5.0 to 128.0 pmol/L

a Within-Laboratory (Total) variability contains within-run, between-run, and between-day variance components

Conclusion: Representative immunoassays utilizing CMIA technology on the Alinity i system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT immunoassays.

A-084

Correlation between Kappa Prozone Effect and IgA Kappa M-proteins in Serum Free Light Chain Assay

K. A. Turner, L. L. Eckelkamp, S. J. Kalass, A. J. Popp, M. R. Snyder, D. L. Murray, M. A. V. Willrich. *Mayo Clinic, Rochester, MN*

Background: Serum free light chain (FLC) assays have been incorporated into diagnostic criteria for multiple myeloma. Testing is subject to analytical issues such as lot-to-lot variation, antigen excess, and non-linearity. A recent lot change for kappa FLC (The Binding Site, Birmingham, UK) prompted physician phone calls questioning normal kappa results in patients with previous history of increased concentrations, triggering laboratory investigations. Prozone effect may occur when the antigen (FLC) is present in great excess and impairs immune-complex formation leading to analyte underestimation. **Methods:** To investigate, previous lot-to-lot validations performed on a nephelometric platform (Siemens BNII, Marburg, Germany) were reviewed. Method comparisons were performed between different lots of kappa FLC, two BNII instruments located at different sites and a turbidimetric platform (Optilite, The Binding Site) with residual serum run on previous lots, n=14. Samples characterized with antigen excess (<0.1% of total FLC test volume) were re-analyzed on nephelometric and turbidimetric platforms, n=16, had additional testing for total IgG, IgA, and IgM (Siemens Healthineers), serum immunofixation (Sebia Inc., France), and MALDI-TOF-MS (MASS-FIX) preceded by immunopurification with beads specific for IgG, IgA, IgM, kappa, and lambda. **Results:** The last two lot-to-lot validations showed insignificant bias with Passing-Bablok (PB) regression lines of y=1.015x-0.166 and y=1.058x+0.031 and Bland-Altman (BA) mean relative differences of 1.4% and 1.7%. The analysis of previously tested and stored sera ranging from 0.55 to 26.81mg/dL (Kappa FLC Reference Interval (RI) 0.33-1.94mg/dL)

showed minimal bias (PB y=0.95x-0.261 and BA 5.2%) with repeat testing on the current lot. Method comparison between the two BNIIs correlated well (y=0.94x+0.065 and -1.8%). Re-running of the antigen excess samples on the alternative BNII yielded no improvement and none of samples were flagged as greater than the upper limit of the initial dilution's calibration curve and concentrations significantly increased upon further technologist-requested dilutions. The final reported results between the two BNII correlated (y=0.94x+3.342 and 3.4%) and ranged from 3.29 to 1620mg/dL. The turbidimetric FLC method identified 14/16 samples as having antigen excess and reflexed them to additional dilutions. The correlation between the nephelometric and turbidimetric platforms was fair (y=0.64x+6.179 and -18.2%) with results based off different dilutions; however, clinical significance remained the same. Immunofixation revealed 12/16 of the samples contained an IgA kappa M-protein with a median IgA of 2325mg/dL (range 710-5410mg/dL, RI 61-356mg/dL). 3/16 cases had free kappa light chains (LC) migrating in the alpha-2 region. MASS-FIX determined that 2/16 cases had glycosylated kappa LC, which is a risk factor for AL amyloidosis. **Conclusion:** Lot-to-lot variation and instrument-specific analytical issues were ruled out as the root cause to the increased cases of kappa FLC prozone effect. Sample characterization showed a propensity of antigen excess to occur with IgA kappa samples using the BNII dilution protocols. Kappa FLC reagents may have lower affinity for this isotype, kappa LC that are more negatively charged and migrate in the alpha-2 region, or glycosylated kappa LC. Additional measures such as delta checks or use of alternative platforms such as the Optilite can mitigate these rare cases and prevent the release of inaccurate test results.

A-085

Performance Comparison of Two Commercially Available Serum Free Light Chain Assays on the Optilite and Architect Analysers

S. Beck[1], S. Gorrie[1], S. Coughtrie[1], M. Coley[2], F. Murphy[2]. *[1]University Hospitals Birmingham, Birmingham, United Kingdom, [2]The Binding Site, Birmingham, United Kingdom*

Background: The role of serum free light chain (FLC) testing has become well-established in the diagnosis, monitoring and response assessment of patients with monoclonal gammopathies (MG). In 2014 the International Myeloma Working Group identified a role for the Freelite FLC assay as a myeloma defining event, based on an involved FLC ≥100mg/L and involved/uninvolved FLC ratio ≥100. More recently, Dejoie proposed that the assay becomes the gold-standard for response assignment in patients with light chain multiple myeloma (LCMM). With an increased reliance on the test, here we assess the performance of two serum FLC assays, Freelite (Optilite) and Diazyme (Architect) and comment upon their comparability.

Methods: Assays were run according to manufacturer's recommendations. 20 blood donor samples without evidence of monoclonal protein by serum protein electrophoresis (SPE) or immunofixation (IFE) were used to validate reference ranges. 91 patients with MG were assessed (3κ and 13λ AL amyloidosis, 18κ and 17λ intact immunoglobulin MM, 8κ and 6λ LCMM and 26 Waldenstrom's macroglobulinaemia). Additionally, 19 samples randomly selected during routine patient analysis were evaluated. Interference was assessed according to CLSI EP07-A2 by challenging the assays with pools of IIMM patient sera.

Results: In blood donor samples, correlation analysis demonstrated proportional differences for κFLC (y=0.8042x+3.92mg/L, R^2=0.4968) and systematic differences for λFLC (y=0.6247x+3.71mg/L, R^2=0.6348) measurements; recovery of λFLC by the Diazyme assay was lower than with Freelite, and decreased with increased concentrations.

In all samples, there was moderate correlation between the κ assays (y=0.7592x+140.16mg/L, R^2=0.6829). Notable systematic differences were noted in IgGκ and κLCMM patients, with significant over- and under-recovery of κFLC, respectively, by the Diazyme assays compared to Freelite. There were three κ samples (2 AL amyloidosis, 1 MM) with gross κFLC underestimation by Diazyme; all three samples had monoclonal FLC bands detectable by electrophoresis. The Diazyme λ assay tended to over-estimate the λFLC value compared to Freelite (y=1.6837x-228.69mg/L, R^2=0.9175). We observed systematic over-recovery of λFLC by the Diazyme assay in IgGλ patients, and under-recovery in AL amyloidosis and λLCMM patients.

The manufacturers reference range for the κ/λ sFLC ratio was validated for Freelite, however we were not able to validate the manufacturers range for Diazyme (results fell outside of the range for 10% of blood donor samples). Five MG patient samples had discrepant ratios; 4 were abnormal by Freelite but normal by Diazyme and 1 was abnormal by Diazyme but normal by Freelite. There was no significant interference with either assay from any of the IIMM patient pools tested.

Conclusion: There was poor quantitative agreement between Diazyme and Freelite κ and λ FLC assays for normal and monoclonal samples, highlighting that the assays are not equivalent. Underestimation of electrophoresis-positive FLC results by the Diazyme assay is a concern, and somewhat surprising given the assays both use polyclonal immunoglobulins as the basis for their test. The assays cannot be used interchangeably, and the guidelines built on Freelite performance must not be assumed for the Diazyme assay. However, further clinical studies are required to establish the clinical performance and value of the new assay.

A-086

Abbott Alinity ci Calibrator/Control Automation Features and Workflow Improvement

R. I. Hong[1], M. L. Bhandari[1], X. Wang[2], C. R. Carlson[1], V. Kulasingam[2]. *[1]Abbott Laboratories, Abbott Park, IL, [2]University Health Network, Toronto, ON, Canada*

Background:

The Alinity ci calibrator and Quality Control (QC) automation features allow for direct sampling from barcoded calibrator and QC vials positioned in specifically designed QC carriers, track the on-board and off-board in-use stability duration and offer on-board 2-8 °C storage capabilities. These features also allow end users to upload lot number and concentration information, improve workflow and reduce the potential for calibrator/QC material contamination and data entry errors within the core laboratory by requiring less manual manipulations.

Objective:

The objectives were: 1) assess calibrator and QC on-board and off-board in-use claims for the Alinity ci system; 2) confirm usability on a subset of high-use assays at University Health Network, Toronto, Canada; and 3) compare Alinity ci calibrator and QC automation workflow to the existing workflow of the ARCHITECT system.

Method:

For calibrators and QC stored on-board the Alinity ci system, percent shift values were determined between day 0 and various on-board test dates (days 3 to 7) to assess on-board stability durations. Acceptable on-board shifts were defined based on assay bias and precision requirements.

For calibrators stored off-board the instrument, additional evaluations were performed to determine in-use stability durations between time 0 and various open vial room temperature time points (1-24 hours). For these evaluations acceptable in-use performance was defined as QCs remaining within pre-determined ranges.

Architect and Alinity ci calibrators and QCs were tested following their respective procedures and overall workflow durations were compared.

Results:

Acceptable on-board percent shifts were observed for 23 clinical chemistry calibrators, 32 immunoassay QCs and 98 clinical chemistry QCs up to 7 days. Data from an external laboratory, University Health Network, confirmed the on-board storage durations of 3 to 7 days for 12 clinical chemistry calibrators, 3 immunoassay QCs and 13 clinical chemistry QCs.

Acceptable off-board in-use performance was observed for 68 clinical chemistry calibrators and 91 immunoassay calibrators up to 24 hours.

Comparison of workflow activities such as calibrator and QC ordering, vial labeling, manual sample pipetting and sample loading demonstrated a 64% reduction in work time when using the Alinity ci system versus the ARCHITECT system.

Conclusion:

The Alinity ci system offers automation features for 182 calibrators and 130 QCs while maintaining acceptable QC performance. These features also improve the core laboratory workflow by significantly reducing preparation time.

A-087

Determination of EBV IgG/IgM Antibodies using a Fully Automated Chemiluminescent Multiplex Analyser System

Z. L. Zhi, C. Dannheimner, S. Amin, C. D. Budd, R. H. Budd. *Dynex Technologies, Inkberrow, United Kingdom*

Background

The detection of Epstein-Barr virus (EBV) viral capsid antigen (VCA) IgG, EBV nuclear antigen (EBNA) IgG, EBV early antigen (EA) IgG, EBV VCA IgM and heterophile IgM in human serum is of value for diagnosing the infectious stages of an EBV

infection. We report here a multiplexed assay based DYNEX® Technologies' fully automated Multiplier® system with a chemiluminescent readout in an ELISA-like assay format for the simultaneous determination of five antibodies associated with EBV.

Materials and Methods

Antigens specific to the aforementioned antibodies were obtained from commercial suppliers. 2mm polystyrene beads coated with individual antigens were embedded into each well of a proprietary 96-well plate. Serum samples were pre-diluted in phosphate-buffered sample diluent and were pipetted into each assay well. After incubating the plate at 37°C for 30 minutes, wells were washed 4 times, and a diluted conjugate of anti-human IgG-horseradish peroxidase (HRP) (or anti-IgM-HRP for IgM detection) was pipetted into each well and the plate incubated for 30 minutes. After a second wash, chemiluminescent substrate was added and the luminescent signal read using the on-board CCD camera. The output data was processed using built-in software and was normalized against pre-defined calibrators for each specificity. Each EBV IgG assay well contains 3 EBV IgG targets: VCA P18, EBNA-1 and EA-D. Each EBV IgM assay well contains 2 IgM targets: VCA GP125 and heterophile antigen.

The precision of the assays was studied by assaying 45 samples in duplicate. The agreement was confirmed by comparing the assay results with 510k-cleared assays.

Results

Precision: The mean percentage coefficient of variation (CV%) for all 3 IgG and 2 IgM assays for 45 samples analysed in duplicate was: VCA P18 IgG, 2.5%; EA-D IgG, 3.9%; EBNA-1 IgG, 4.9%; GP125 IgM, 3.6%; Heterophile IgM, 4.7%.

Correlation with the predicate index data: VCA P18, R^2=0.80; EA-D, R^2=0.90; EBNA-1, R^2=0.94; GP125, R^2=0.83.

Positive and negative agreement against predicates: VCA P18, 100% (40/40) / 80% (4/5); EA-D, 100% (26/26) / 100%(19/19); EBNA-1, 100% (32/32) / 100%(13/13); GP125, 96.0% (24/25) / 84.2% (16/19); Heterophile, 95.5% (21/22) / 100% (22/22).

Test with SeraCare EBV IgG/IgM performance panel samples: VCA P18 IgG, R^2=0.90; EA-D IgG, R^2=0.82; EBNA-1 IgG, R^2=0.93; GP125 IgM, R^2=0.987; All against commercial 510k approved ELISA assays. Heterophile IgM against Sure-Vue™ latex assay, positive agreement 100% (7/7), negative agreement 85.7% (12/14).

Conclusions

This multiplexed assay provided reproducible semi-quantitative results for 5 EBV-related antibodies (3 IgG and 2 IgM) in serum samples. The Multiplexed format is ideal for clinical applications, as it can handle up to 92 test samples within a single plate in a fully automated manner. When 2 plates are run together, 460 results of EBV IgG/IgM are generated in 2.5 hours.

Under development. The performance characteristics of this device have not been established. Not available for sale, and its future availability cannot be guaranteed. The Multiplier is currently Research Use Only.

A-088

Reference Range for IgA, IgM, IgG in Healthy Children and Adolescents in the City of Cuiabá - Brazil

N. Slhessarenko[1], M. E. Slhessarenko[2], M. C. Freire[3], C. J. F. Fontes[4], P. R. Telles-Dias[5], R. S. Azevedo[6], A. Andriolo[7]. *[1]DASA, Cuiabá, Brazil, [2]Faculdade Israelita de Ciências da Saúde Albert Einstein, São Paulo, Brazil, [3]DASA, Rio de Janeiro, Brazil, [4]Federal University of Mato Grosso School of Medicine, Cuiabá, Brazil, [5]Universidade do Estado do Rio de Janeiro, RIO DE JANEIRO, Brazil, [6]University of São Paulo School of Medicine, São Paulo, Brazil, [7]Federal University of São Paulo−Unifesp, São Paulo, Brazil*

Background: As many as 70% of medical decisions are based on laboratory results, thus establishing laboratory's own Reference Intervals (RI) is reccomended, as it reflects characteristics of the population where the tests will be applied to. Unfortunatelly, it is not frequent, as it is an expensive and time-consuming task. As a consequence, RI's regional, ethnic and socioeconomic variations are not taken into account in many test reports. When it comes to pediatric RI, the gap is even larger. **Methods**: To determine RI for immunoglobulin (IG) in a Brazilian city among healthy children from 1 to 13 years incompleted, 1,866 scholars were randomly submitted to data collection and laboratory testing. Subjects presenting at the time of collection without any known underlying disease, clinical signs or symptoms, health complaints and medication use were included in the study. Statistical analysis was performed using "R" software. The sample calculation was based on the Clinical and Laboratory Standards Institute document, variance homogeneity in the age groups was analyzed using Bartlett's test. Then, the analysis of variance (ANOVA) test was used for homogeneous variances and Kruskal-Wallis test was used for non-homogeneous variances. Subsequently, the Bonferroni post hoc test was used, which adjusted the level of significance for multiple comparisons in 2 by 2 groups, which allowed grouping the age ranges. Once grouped into age ranges for IG, Bartlett's test was repeated, and the ANOVA or Kruskal-Wallis tests were applied. While keeping the age groups, outliers were excluded by calculating the mean plus or minus three standard deviations. After outlier exclusion, the distribution was carried out in percentiles. The level of significance was set at 5% (alpha = 0.05). Superior reference value was determined by the result of the 95th percentile and inferior reference value by the 5th percentile. IG was determined by nefelometry. **Results**: Inferior and superior limits (mg/dL) and age groups found were the following. For IgA: 24.30 and 141.23 for 1 year old (yo); 21.98 and 178.87 for 2-4 yo; 27.35 and 223.20 for 5-6 yo; 33.27 and 267.36 for 7-12 yo. For IgM: 30.71 and 205.48 for 1-2 yo; 21.86 and 200.23 for 3-7 yo and 26.56 and 217.28 for 8-12 yo. For IgG: 706.73 and 1715,67 for 1-6 yo and 824.11 and 1870.17 for 7-12 yo. **Conclusion**: The study successfully determined age groups and RI for IgA, IgM and IgG for children and adolescents for a multiethnic population from a Brazilian city. These findings may better represent RI for the Brazilian pediatric population than those currently in use, contributing to improve diagnostics in this country.

A-089

The Magnesium Enzymatic Assay (LN 3P68) is Now Available for use Worldwide on the Abbott ARCHITECT cSystems

K. Bachhawat, K. Landuyt, J. King, B. Nance, J. Salazar, M. Beischer. *Abbott Laboratories, Irving, TX*

OBJECTIVE: To present performance and interference test results for the Abbott ARCHITECT Magnesium **Enzymatic** (MAG, List Number [LN] 3P68) assay for use with serum, plasma, or urine on the Architect *c*Systems.

RELEVANCE: The MAG (LN 3P68) assay is liquid, ready-to-use, and measures Magnesium in serum, plasma or urine. Magnesium is an essential nutrient involved in many biochemical functions. It has a structural role in nucleic acids and ribosomal particles, is required as an activator for many enzymes, and has a role in energy producing oxidative phosphorylation.

METHODOLOGY: The magnesium present in a patient sample functions as a cofactor for isocitrate dehydrogenase (available in excess) in an enzymatic reaction. Thus, the rate of increase in absorbance at 340 nm, due to the formation of NADPH, is directly proportional to the magnesium concentration:

Isocitrate dehydrogenase

D-isocitric acid+ NADP -------------------> 2-Oxoglutarate + CO2 + NADPH

Mg+2

VALIDATION: Table 1 displays the MAG (LN 3P68) assay performance characteristics, the highest acceptable interference levels for two magnesium concentration ranges, and the method correlation relative to a commercially available predicate magnesium assay.

CONCLUSIONS: The MAG (LN 3P68) assay is liquid, ready-to-use, comparable to a commercially available method, and can be used for measuring magnesium in serum/plasma or urine worldwide.

Table 1. Performance characteristics, interference and method comparison data, for the MAG (LN 3P68) assay.

Performance Characteristics for the Enzymatic Magnesium (LN 3P68) Assay			
Sample Type	Serum, plasma and urine		
Limit of Quantitation (mg/dL)	0.16 (serum/plasma) 0.79 (urine)		
Measuring Interval (mg/dL)	0.60 to 9.50 (serum/plasma) 1.81 to 26.35 (urine)		
On-Board Stability Calibration Stability	30 days 30 days		
Interferences – Highest Acceptable Interferent Levels			
Interferent	**Low Serum Magnesium Conc. (1.9 to 2.1 mg/dL)**		**Low Urine Magnesium Conc. (4.3 to 5.2 mg/dL)**
Conjugated Bilirubin	55.3 mg/dL		59.9 mg/dL
Unconjugated Bilirubin	60.3 mg/dL		n/a
Hemoglobin	250 mg/dL		1200 mg/dL
Human Triglycerides	3647 mg/dL		n/a
Intralipid	2476 mg/dL		n/a
Calcium	28 mg/dL		26 mg/dL
Study		**MAG (y) vs. Commercially Available Predicate (x)**	**Abbott ARCHITECT Intraplatform *c*System**
Serum Method Correlation	**N** **R** **Equation** **Range (mg/dL)**	122 0.9979 y = 0.95x - 0.02 0.65-9.00	134 0.9997 y = 1.00x - 0.05 0.68 – 9.35
Urine Method Correlation	**N** **R** **Equation** **Range (mg/dL)**	118 1.000 y = 1.04x – 0.10 1.82 – 23.25	174 0.9988 y = 1.04x – 0.06 1.91 – 23.72

A-090

The Intensity of the Quantiferon-Monitor and Quantiferon-CMV Interferon-Gamma Production is Directly Related to the Lymphocytes Count in Immunocompetent and Immunocompromised Patients

V. Vidotto Frade, P. Udiloff, D. R. de Souza, L. Rodrigues, C. M. M. Dias, L. Pierrotti. *DASA, Barueri, Brazil*

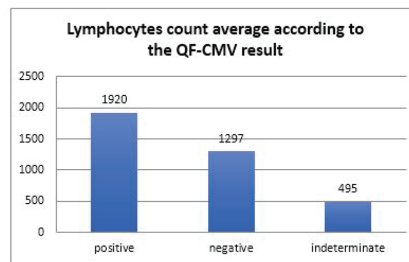
Background: Quantiferon (QF)-Monitor assay is an in vitro diagnostic test for cell-mediated immune function evaluation by measuring interferon-gamma (IFN-γ) in plasma by enzyme-linked immunosorbent assay (ELISA) after plasma incubation with innate or adaptive immune response stimulants. QF-CMV assay has the same principle but is able to quantify the immune cellular responses to CMV. These tests are designed to evaluate the intensity of immunosuppression state, which is particularly useful among immunocompromised hosts such as kidney transplant recipients (KTR). However, both tests are dependent of the lymphocytes counts. This study aim is to evaluate the relation between the QF-Monitor and QF-CMV IFN-γ production and the lymphocytes count in healthy individuals and KTR. **Methods:** QF-Monitor and QF-CMV were performed in 81 samples from 34 healthy subjects and 47 KTR; in addition we performed the complete blood count tests including the lymphocytes count determination and CMV serology. CMV-IgG seronegative patients were excluded from this analysis. For the analysis purpose, values of IFN-γ production in QF-Monitor above 1000 and IFN-γ production in QF-CMV above 10 or less than 0,01 UI/ml were estimated (1001, 11 and 0.005, respectively).**Results:** 77 subjects were analyzed; 2 healthy and 2 KTR CMV-IgG seronegative subjects were excluded; 2 KTR were excluded because lymphocyte counts values were not available. The distribution of QF-Monitor and QF-CMV IFN-γ production results according to the total lymphocytes count group (<500; 500 - 1000; > 1000) are shown in table 1. The average of IFN-γ production results progressively increased according to the lymphocytes count increment. In addition, the lymphocytes average count is higher among the QF-CMV positive individuals (58/75, 77%) compared to those with negative (15, 20%) and indeterminate results (2, 3%), respectively (figure 1).

Conclusion: QF-Monitor and QF-CMV tests evaluate the response to stimulation of the innate immune system and adaptive, directly correlated with lymphocyte count.

Table 1. The average of IFN-γ production results progressively increased according to increment of lymphocytes count.

IFN production average (IU/ml)		Total	Healthy subjects	KTR
Number		75	32	43
QF-Monitor final result	< 500	13.2 (n=6)		13.2 (n=6)
	500 – 1000	124.5 (n=12)		124.5 (n=12)
	>1000	566.9 (n=57)	811.0 (n=32)	231.7 (n=25)
QF-CMV peptide tube - Nil	< 500	0.5 (n=6)		0.5 (n=6)
	500 - 1000	2.5 (n=12)		2.5 (n=12)
	>1000	5.1 (n=57)	4.5 (n=32)	5.8 (n=32)
QF-CMV positive control - Nil	< 500	1.0 (n=6)		1.0 (n=6)
	500 - 1000	5.9 (n=12)		5.9 (n=12)
	>1000	10.0 (n=57)	11 (n=32)	8.6 (n=25)

Figure 1. The lymphocytes average count according to QF-CMV results.



A-091

Performance Evaluation of Plasma Sample Types on the ADVIA Centaur and Atellica IM Analyzers

H. Leipold, K. Thakur, J. Freeman. *Siemens Healthcare Diagnostics, Tarrytown, NY*

Background: Siemens Healthineers reproductive endocrinology assays—Total hCG (ThCG), Leutinizing Hormone (LH), Follicle Stimulating Hormone (FSH), Progesterone (PRGE), and Prolactin (PRL)—are approved for serum specimen types for testing on the ADVIA Centaur® and Atellica® IM analyzers. Adding plasma specimen types to the intended use may give added flexibility in the choice of blood collection tubes. Specimen equivalence studies have been carried out by method comparison with serum, EDTA, and lithium heparin blood collection tubes to qualify additional plasma claims for these assays.

Methods: The specimen equivalence study was carried out for these assays using matched sets of patient samples. The matched sets for each assay (serum, EDTA, and lithium heparin samples) were evaluated with one lot of reagent on two immunoassay instruments: the ADVIA Centaur XP Immunoassay System and Atellica IM Analyzer. Matched donor samples were either native or contrived to span the range for each assay. In addition, EDTA and heparin interferences were tested for all assays at three times the EDTA- or heparin-coated concentration to simulate a short-fill sample as per CLSI guidelines.

Results: The data obtained from the plasma samples on the ADVIA Centaur and Atellica IM analyzers from these studies tended to demonstrate acceptable correlation to EDTA vs. serum and heparin vs. serum samples, with a slope of 0.90-1.10 across all samples for each assay on each platform. EDTA at a spiked concentration of 5.4 mg/mL and heparin at a spiked concentration of 45 U/mL demonstrated less than 10% change in results at low and high concentrations of analyte for each assay.

Conclusion: The performance of plasma samples is in alignment with serum samples, demonstrating equivalent accuracy for the reproductive endocrinology assays listed above based on these studies. The study is ongoing for other assay groups, including cancer markers (CA15-3, CA125II, carcinoembryonic antigen, BR27.29, free prostate-specific antigen, prostate-specific antigen, complexed prostate specific antigen, alpha fetoprotein), thyroid function (free triiodothyronine, total triiodothyronine, total thyroxine, thyroid uptake), metabolic function (cortisol), TDM (digoxin), and allergy (total IgE).

A-092

Sensitization to Common Inhalant and Food Allergens in West South-Central Region of United States

D. Ponds, S. Mansani, L. Romero, S. Alsaab, M. Haidari. *Elite Medical Laboratory Solutions, Tomball, TX*

Background: Allergic sensitization is the presence of allergen-specific serum immunoglobulin E against food or inhalant allergens in body of tested individuals. It is generally agreed that sensitization to allergens is an important risk factor for allergic diseases such as asthma, rhinitis, and eczema. Prevalence of sensitization varies between countries and between geographic regions of a country. No previous studies explored the common inhalant and food allergens in West South-Central region of United States. This study was conducted to determine the prevalence of sensitization to inhalant and food allergens in Texas, United Sates.

Methods: The results of 1,074 patients` allergy tests that quantified the specific IgE against 72 inhalant and food allergens between August 2017 and December 2018 were evaluated in a retrospective study. All specimens were collected from primary care clinics at Texas and the measurement was performed by Immulite 2000 XPI autoanalyzer using 3gAllergy™ assay (Siemens). The Levels of IgE for a particular allergen was divided into semiquantitative classes as absent (class 0, < 0.1), very low (class 0, 0.1- 0.34 KU/L), low (class I, 0.35-0.69 KU/L), moderate ((class II, 0.7- 3.49 KU/L), high (class III, 3.5-17.49 KU/L), and very high (classes IV to VI >17.5 KU/L). The percentage of positive tests and the class responses were identified.

Results: The patients were 356 males and 718 females with the mean age of 43 ± 14 years old. The most common sensitization for inhalant allergens with the levels of moderate or higher classes were dust mites (dermatophagoids, 23%), different types of grasses (16%), cat-dander epithelium (16%), cedar tree (11%), house dust greer (11%) and dog dander (10%). Prevalence of sensitization to food allergens was significantly lower than inhalant allergens. Among 27 studied food allergens only one allergen had a frequency of greater than 5 % in moderate or higher classes of IgE level. While among 45 inhalant allergens 27 had a frequency of greater than 5% in moderate or higher classes of IgE level. The levels of specific IgE against all assayed food allergens were classified as low with a maximum prevalence of 10%, or moderate to higher levels with a maximum prevalence of 5%. The most common sensitization for food allergens was pecan with a frequency of 3% in low level or less and 6% for moderate to higher levels of IgE.

Conclusion: The most frequent sensitization to allergens with moderate or higher classes of IgE levels among Texans are dust mites (dermatophagoids), different types of grasses, cat -dander epithelium, cedar tree house, dust greer, and dog dander. In comparison with inhalant allergens sensitization to food allergens was mostly at lower classes and significantly lower frequency.

A-093

Utilization Review of Autoimmune Encephalitis and Paraneoplastic Antibody Panels

A. Jasim, I. Larsen, A. B. Chambliss. *LAC+USC Medical Center/University of Southern California, Los Angeles, CA*

Background: Autoimmune encephalitis (AE) and paraneoplastic syndromes are rare neurologic disorders that are difficult to diagnose clinically, given their variety of neurological manifestations. Recently, comprehensive antibody panels have been introduced to assist in diagnosis, despite low sensitivity and high costs. Our institution implemented approval guidelines to oversee appropriate ordering of these antibody panels. This study evaluated the effect of implementing these guidelines on test request frequency and the influence of the test results on patients' medical management.

Methods: We conducted retrospective chart reviews on patients who were tested with AE/paraneoplastic antibody panels at a reference laboratory for one year before and one year after the implementation of test approval guidelines, which were drafted in collaboration with the departments of Pathology and Neurology. The guidelines detailed test pricing, requirements for patient evaluation by Neurology and reasonable exclusion of alternate symptom etiologies, and possible targeted antibody testing alternatives. Patients' charts were reviewed to determine whether alternate causes of symptoms were reasonably excluded prior to testing and whether test results influenced medical management. Results from both years were compared to assess the influence of implementing the approval guidelines. This study was approved by the Institutional Review Board of the University of Southern California.

Results: AE/paraneoplastic antibody panels were ordered for 11 patients in 2017, prior to guideline implementation, and for 14 patients in 2018, after guideline implementation, in either serum, CSF, or both. Prior to guideline implementation, only 6/11 (55%) patients had alternate etiologies reasonably excluded prior to testing. Prior to guideline implementation, 3/11 (27%) patients had detectable antibodies, and final diagnosis was influenced from the test results in 1/11 (9%) patient; however, medical management was not altered for that patient, as intravenous immunoglobulin had already been initiated. After guideline implementation, 4/14 patients (29%) had detectable antibodies, and final diagnosis and medical management was influenced from tests results for 2/14 (14%) patients.

Conclusion: Frequency of sent test orders for AE/paraneoplastic antibody panels increased from 2017 to 2018, despite implementation of test approval guidelines in the second year, likely owing to increased awareness of test availability. Our results suggest that upon implementation of test approval guidelines, ordering physicians were more likely to first exclude alternate causes of symptoms, thereby decreasing the frequency of inappropriate test requests and minimizing send-out costs. If antibodies are detected, these panels may help guide therapy in some patients. However, because of low sensitivity and high costs, it is important to carefully examine test requests.

A-094

Evaluation of Immunologic Methods to Detect *Leishmania donovani*

F. L. O. Marinho[1], D. Zauli[1], F. Brito[2]. [1]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil,* [2]*Hermes Pardini Institute, Belo Horizonte, Brazil*

Background: Human visceral leishmaniasis (VL) is a lethal form of leishmaniasis caused by Leishmania donovani complex. Serological tests, like ELISA and indirect immunofluorescence (IFAT), are the least invasive diagnostic tools, but their reliability was compromised due to cross reactivity with other disease conditions. On the other hand, recombinant K39 antigen used either is an immunochromatographic or an ELISA format, is more sensitive and specific than IFAT and ELISA using crude antigen, and a valuable tool in validating diagnostic methods for VL. **Objective**: To estimate the agreement, sensitivities and specificities of two different IFAT diagnostic tests for VL (Euroimmun and Biognost), using the IT LEISH immunochromatographic K39 test (Bio-Rad) as the gold standard. **Methods**: The IFAT tests were evaluated in 94 negative and 18 K39 positive samples. **Results:** The Kappa index was 0.590 (Biognost and Euroimmun, 95% CI= 0,501 to 0,679); 0.485 (Biognost and K39, 95% CI= 0.313 to 0.657), and 0.676 (Euroimmun and K39, 95% CI= 0.491 to 0.861). The sensibility and specificity of Biognost and Euroimmun tests was 94.1%/75.9% and 82.4%/91.6%, respectively. **Conclusions**. Despite a good level of agreement between the IFAT tests, the observed differences in sensitivity and specificity suggests a lack of standardization among the commercial tests available for the serological diagnosis of VL, and points out the need to use a reference method for evaluating the diagnostic performance of these tests.

A-095

Low Serum TFF1 is Associated with Aggressive Breast Cancer Variants and Poor Prognosis of Breast Cancers

J. Yi[1], L. Ren[2], D. L[1], J. Wu[1], J. Wang[2]. [1]*Peking Union Meical College Hospital, Beijing, China,* [2]*The State Key Laboratory of Bioactive Substance and Function of Natural Medicines; Beijing Key Laboratory of Drug Target Research and Drug Screen, Institute of Materia Medica, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China*

Background: BC (breast cancer) remains a common malignancy in women. It is very difficult to predict prognosis of BC for its high heterogeneity. Serum TFF1 has been shown to be correlated with breast cancer. However, associations between TFF1 expression and the characteristics,the prognosis of BC are still unclear. The aim of this study is to assess if the TFF1 is used to predict prognosis of patients with BC.

Methods: In silicon assay was carried out to investigate if TFF1 expression was associated with clinical characters and survival rate of breast cancer patients. TFF1 in serum of 70 BC patients and 32 healthy controls were measured by ELISA assay.

Results: Results showed that TFF1 mRNA expression was correlated with expression of luminal cancers signatures: ESR1, GATA3, FOXA1, MYB and XBP1. Importantly, there was higher expression of TFF1 in BC patients with ER^+ than that in BC patients with ER^- while there was lower expression of TFF1 in TNBCs than the other types breast cancers. In addition, expression of TFF1 was decreased as development of breast cancers, especially in breast cancers at Grade 3($P<0.05$). Furthermore, serum TFF1 was statistically different among the status of ER, PR and HER2 (P=0.04139, 0.0018, 0.0004). Elevated TFF1 concentration in serum was found to be correlated with increased overall survival (P =0.00068).

Conclusion: All these results suggest that TFF1 level in serum may be associated with prognosis of patients with breast cancers.

A-096

Rapid Assessment of Drug Resistance of *Mycobacterium tuberculosis*

R. Takeuchi[1], Y. Murase[2], Y. Ohtaki[1], K. Nakaishi[1], S. Mitarai[2], E. Ito[3], S. Watabe[1]. [1]*TAUNS laboratories Inc., Izunokuni-shi, Shizuoka, Japan,* [2]*Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Kiyose-shi, Tokyo, Japan,* [3]*Waseda University, Wakamatsu-cho, Tokyo, Japan*

Background: In a tuberculosis (TB) treatment, a prompt assessment of drug-resistance is desired. Although nucleic acid tests can get a result in a short time, their judgement intrinsically relies on known resistance mechanisms so that a misjudgment occurs when tubercle bacilli hold an unknown or newly emerging resistance mechanism. Reliable culture-based phenotypic drug resistance tests require a long inspection time due to the very slow growth rate of *Mycobacterium tuberculosis*. Recently, we have developed an ultrasensitive ELISA to detect proteins at level of 10^{-20} moles/assay by use of thio-NAD cycling. Here, we applied ultrasensitive ELISA to detect a trace amount of MPB64, a specific protein secreted from active tubercle bacilli, and this assay may enable us to assess the effect of drugs on tubercle bacilli in a short time by capturing a MPB64 secretion amount as a proxy of activity of active tubercle bacilli.

Methods: Fresh culture of H37Rv strain was harvested and washed three times to remove MPB64 secreted during the incubation. The washed culture was adjusted for concentration (OD_{530} = 1.8 x 10^{-3}), then equally dispensed to serial concentrations of 8 types of anti-TB drugs (BrothMIC MTB, Kyokuto, Japan), which are commonly used in medical sites in Japan. After 24 hour incubation at 37°C, tubercle bacilli were removed by 0.1 μm centrifugal filter units, and the firtrate was used as a sample. MPB64 concentrations of samples were determined by the ultrasensitive sandwich ELISA. Two specific mouse monoclonal antibodies for MPB64 were prepared for the sandwich ELISA, one of which was conjugated with alkaline phosphatase (ALP) and the other was immobilized on a microplate.

Results: Before experiments, we confirmed that MPB64 degradation did not occur during the incubation. The MPB64 concentration of a control sample (without drug) was increased to 747 pg/mL (SD 57) after 24 hour incubation. In the presence of various drugs, these MPB64 concentrations were varied from 15 to 1231 pg/mL, and the dose response curves seemed to be different according to the different mechanisms of drugs. Six out of 8 drugs (SM, KM, REP, PBT, LVFX and CPFX) decreased MPB64 secretion, whereas the other 2 drugs (EB and INH) increased MPB64 secretion.

Conclusion: We successfully detected clear response of tubercle bacilli cells to the drugs tested by just 1-day incubation. This is because our ultrasensitive ELISA for MPB64 detection is highly sensitive, which can detect a few hundreds CFU/mL of tubercle bacilli. Our ultrasensitive ELISA is a low-priced and user-friendly without any specialized apparatus. We believe that our assay becomes a reliable rapid test of TB drug resistance.

A-097

Mycoplasma Pneumonia Antigen Detected by Ultrasensitive ELISA with thio-NAD Cycling

m. komatsu[1], K. Nakaishi[1], T. Yoshimura[2], E. Ito[3], S. Watabe[1]. [1]*Tauns laboratories ,Inc., Izunokuni, Japan,* [2]*Health Sciences University, Ishikari,Hokkaido, Japan,* [3]*Department of Biology,Waseda University, Shinjuku Tokyo, Japan*

Background: *Mycoplasma pneumoniae* (MP) is a type of bacteria which can cause respiratory diseases in humans. In the medical site, a rapid and accurate diagnosis of Mycoplasma pneumonia at early stage of symptoms has been required, because prompt medication to a patient holding a low number of bacteria prevents aggravation of symptoms and secondary infection. The conventional tests do not meet the requirements: serum antibody tests are insufficient sensitivity and require paired serum, and nucleic acid tests require long inspection time and an expensive instrument. Although lateral flow tests for MP antigen have placed on the market recently, its insufficient sensitivity has been pointed out so far. Here, we report a highly sensitive and rapid method for detection of MP antigen developed by applying an ultrasensitive ELISA method based on thio-NAD cycling, which were developed by ourselves.

Methods: In the sandwich ELISA, two specific antibodies for MP antigen were used, one of which was conjugated with alkaline phosphate (ALP). An androsterone derivative with a phosphate was hydrolyzed by ALP, and this derivative was then employed in the enzyme cycling. MP antigen could be determined by the accumulated amount of thio-NADH in the enzyme cycling. In this report, cultures of M129 strain and FH strain were used as the antigen.

Results: We succeeded in detecting cultured cells of FH strain at the level of 20 cfu/mL. When the results using the antigen of M129 strain and FH strain obtained in our ultrasensitive ELISA were compared with those using a conventional *p*NPP method and a commercially available MP antigen measuring kit, the sensitivity was found to be about 150-fold improvement and about 80-fold improvement, respectively.

Conclusion: The present results showed that our ultrasensitive ELISA can be used enough to detect a trace amount of MP antigen in a short time. We believe that this ultrasensitive mycoplasma MP antigen assay method is useful for the early diagnosis of MP.

A-098

JAK/STAT Signaling is Involved in IL-35 Induced Inhibition of HBV Antigen Specific CTL Exhaution in Chronic Hepatitis B

Y. Dong. *Key Laboratory of Clinical in Vitro Diagnostic Techniques of Zhejiang Province, Hangzhou, China*

Background: IL-35 is composed of P35 and EBI3. As a new member of the IL-12 family, IL-35 can significantly inhibit the proliferation of HBV-specific CTLs and the function of effector T cells. IL-35 has been reported to possibly inhibit the proliferation and differentiation of HBV-specific CTLs, upregulate the expression of exhaustion-associated molecules or downregulate the expression of effector molecules through the JAK/STAT pathway, ultimately resulting in exhaustion of HBV-specific CTLs.Methods: Using western bolt, the expression levels of JAK-STAT pathway on CTL were analyzed . The ability of cytokines secretion such as IFN-γ and TNF-α of CTL were also analysed by flow cytometry.Results: Our results show that IL-35 can activate the JAK1/TYK2-STAT1/STAT4 pathway in CTLs in vitro. IFN-γ and TNF-α expression was increased in CTLs in the presence of a JAK-STAT pathway blocker. In addition, we evaluated the expression of the exhaustion-associated molecules PD-1, CTLA-4 and LAG-3 in CTLs after adding the JAK-STAT blocker. The results showed that the expression of exhaustion-associated molecules on the CTL surface was decreased after blocking the JAK-STAT pathway.

Conclusion: IL-35 inhibits the function of HBV-specific CTLs through the JAK1/TYK2-STAT1/STAT4 pathway. After blocking the JAK-STAT pathway, the secretory function factors IFN-γ and TNF-α in CTLs recovered, and the expression of exhaustion-associated molecules decreased. Our results suggested that the function of CTLs was recovered after blocking the JAK-STAT pathway. The data provide a new experimental basis for immunotherapy for chronic hepatitis B.

A-099

Analysis of Serum Cytokine Profiles in Korean Patients with Systemic Lupus Erythematosus

E. Oh[1], J. Park[1], W. Jang[1], S. Kwok[2], S. Park[2]. [1]*Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of,* [2]*Division of Rheumatology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of*

Background: Systemic lupus erythematosus (SLE) is a highly complex and heterogeneous autoimmune disease with a broad clinical and immunological manifestation. Development of relevant biomarkers for understanding this heterogeneity and predicting the treatment response is importantly needed in SLE patients. The purpose of this study was to investigate the role of serum cytokines as surrogate markers to stratify disease activity and lupus nephritis (LN) in SLE patients.

Methods: We measured the levels of twelve cytokines using sensitive multiplex bead assays and analyzed the associations with specific SLE disease features including disease activity, LN, and laboratory markers. Cytokine panel assay including interleukin (IL)-2, IL-6, IL-8/CXCL8, IL-10, IL-12p40, IL-17, IL-18, interferon (IFN)-γ, macrophage inflammatory protein (MIP)-1α/CCL3, MIP-1β/CCL4, (RANTES)/CCL5, and tumor necrosis factor (TNF)-α was performed in 203 SLE patients with or without LN (age: 34 years; 91% female) recruited between January 2010 and May 2012. Clinical features and laboratory data relevant to the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE were retrieved retrospectively from medical records. Disease activity was determined by Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K) and active disease was defined as SLEDAI-2 K > 4.

Results: Four cytokines including IL-10, IL-12, IFN-γ, and TNF-α were correlated with disease activity and/or LN by Mann-Whitney U-test. Among them, IL-10 and TNF-α were associated with both disease activity and LN (P < 0.05). Laboratory markers including C3, C4, hsCRP, ESR, anti-dsDNA, and anti-C1q levels were also significantly different according to the presence of disease activity (P < 0.05). However, by multivariate logistic regression analysis, serum cytokines were not retained and serum anti-dsDNA and anti-C1q levels were only associated with disease activity in SLE patients (P < 0.05). Similar with comparison results of disease activity, high serum cytokine levels such as IL-10, IL-12, and TNF-α, low complements such as C3 and C4, high levels of anti-dsDNA and anti-C1q were statistically different between without and with LN in SLE patients (P < 0.05). By multiple regression analysis to detect independent associations with LN, anti-dsDNA, active SLE with SLEDAI-2 K > 4, and anti-Sm were significant correlates of LN in SLE patients.

Conclusion: Our study demonstrated that four cytokine levels of IL-10, IL-12, IFN-γ, and TNF-α could be surrogate markers for assessing disease activity and the presence of LN. In addition, positive correlations between these cytokines suggest that these cytokines in combination with complement, anti-dsDNA, anti-C1q, and anti-Sm may be helpful for monitoring SLE disease activity and/or LN in SLE.

A-100

Evaluation Reports of External Quality Controls for Allergen Specific Immunoglobulin E Tests in Korea

H. Lee[1], J. Ryu[2], A. Choi[2], E. Oh[2]. *[1]Department of Laboratory Medicine, International St. Mary's Hospital, College of Medicine, Catholic Kwandong University, Incheon, Korea, Republic of, [2]Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of*

Background: The incidence of allergic conditions has increased over the last several decades and detection of allergen specific IgE is meaningful for the diagnosis and management of diseases. Still, there is no standardized reference method for specific IgE detection, and external quality assessment (EQA) is important to determine the reliability and comparability between clinical laboratories. Since 2012, we continuously conducted EQA for specific IgE tests in Korean clinical laboratories using the commercial multiple allergen simultaneous test (MAST) methodology. To date, EQA has been conducted 13 rounds in total, and we analyzed the reported overall results.

Methods: Pooled sera were collected form allergy patients and were sent to laboratories two times per year. EQA panel included 2-3 samples concerning allergen-specific IgE antibodies to 30-50 specificities and asked results of total IgE and classes. The acceptable range was set based on +/- 1 class of the reported results by more than 90% of the responding laboratories.

Results: The number of participating institution has been gradually increased from the 61 laboratories in initial to 89 laboratories in 2018. The average response rate was 97.2 +/- 2.9%. Of the 1461 valid total IgE tests, 99.9% (1460) were acceptable. Of the overall 33,873 antigen specific IgE tests, 99.4% of the results met the acceptance criteria, while 0.2% showed a deviation of one MAST class, 0.4% showed more than 2 classes, respectively. The false negative specific IgE results were significantly frequent than false positive antigen-specific IgE levels (0.6 vs. 0.05%, P<0.001). We divided allergens into following subgroups as food allergens (n=13,156), pollen (11,418), mites (4,331), mold (n=2,686) and animal epithelia (n=2,282). The each allergen groups yield excellent results with less than 1.0 % of false report and there were no significant differences in false results (0.7% for foodstuffs, 0.6% for pollen, 0.5% for mites, 0.6% for mold, 0.7% for animal epithelia).

Conclusion: There were good agreement and comparability for the detection of allergen specific IgE between the individual laboratories in Korea. It is necessary to perform EQA continuously to improve the accuracy and quality of laboratories.

A-101

Comparison of Cytotoxicity and IFN-gamma Production Methods for Measuring Reduced Natural Killer Cell Function for HLH Diagnosis

H. Ryu[1], H. Kim[2], J. Lee[2], K. Park[2], A. Choi[2], J. Yoon[3], E. Oh[2]. *[1]Department of Laboratory Medicine, Samkwang Medical Laboratories, Seoul, Korea, Republic of, [2]Department of Laboratory Medicine, The Catholic University of Korea, Seoul, Korea, Republic of, [3]Department of Hematology, The Catholic University of Korea, Seoul, Korea, Republic of*

Background:

Hemophagocytic lymphohistiocytosis (HLH) is an immune dysregulation disorder characterized by impairment of cytotoxic activity of T lymphocytes and natural killer (NK) cells. Low or absent NK cell activity is one of the diagnostic criteria of HLH, which can be measured in various methods. However, there is no definite standard method or clear cut-off value for NK cell function measurement. In this study, we compared NK cytotoxicity flowcytometry-based assay (NK-cytotoxicity) and NK cell-specific IFN-gamma-releasing assay (NK-IGRA) for HLH diagnosis.

Methods:

We enrolled 138 patients referred for NK-cytotoxicity in the Seoul St. Mary's Hospital from November 2015 to December 2018. Most of them had recent febrile cytopenia and were clinically evaluated for HLH. Both NK-cytotoxicity and NK-IGRA were tested on 347 initial or follow-up samples. NK-cytotoxicity was assessed using Navios flow cytometer (Beckman Coulter, Miami, FL, USA) by calculating 7-AAD positivity of CFSE-labeled K562 cell. NK activity for IFN-γ release was analyzed by enzyme immunoassay using NK Vue Kit (ATgen, Sungnam, Korea). Serum soluble IL-2 receptor (sIL-2r) and lymphocyte subsets (CD3, CD4, CD8, CD16, CD56bright, CD56dim) tests were perfomed.

Results:

Of 138 patients, 74 (54.0%) patients were clinically diagnosed with HLH based on 2004 criteria. NK-cytotoxicity and NK-IGRA results were decreased in HLH patients compared to the non-HLH febrile patients [median (95% CI): 12.4 (10.7 to 14.9) vs. 21.7 (16.2 to 29.7), *P*=0.001] [13.5 (10.0 to 24.0) vs. 39.0 (18.4 to 78.6), *P*=0.002], respectively. Among HLH patients, 68 (91.9%) patients showed decreased NK cytotoxicity (cut-off < 38.5%) and 66 (89.2%) patients showed decreased NK-IGRA level (< 250 pg/ml). The agreement between two assays for qualitative detection was 76.9% in all sample and 71.7% in 138 initial tests for the diagnosis. However, in quantitative comparisons, NK cytotoxicity levels were not correlated with NK-IGRA levels (r=0.057). NK function levels from NK cytotoxicity revealed weak positive correlation with NK cell percent and count from lymphocyte subset results (r=0.259 and 0.252, respectively). sIL-2r levels were significantly increased in HLH patients, however, showed no correlation with NK-cytotoxicity or NK-IGRA level. Using the ROC curve analysis, NK-cytotoxicity (AUC of 0.674 with cutoff ≤33.4%) and NK-IGRA (AUC of 0.617 with cutoff ≤14.6 pg/mL) showed sensitivities of 89.2% and 58.1% and specificities of 48.4% and 65.6%, respectively.

Conclusions:

Our study suggests that both NK-cytotoxicity and NK-IGRA might be used for diagnosis of HLH with appropriate criteria and good qualitative agreement. As there was no correlation with quantitative levels in patients for diagnosis of HLH, further studies might be necessary to understand precise mechanism of NK cell function.

A-102

Comparison of Two Automated Systems for Indirect Immunofluorescent Antinuclear Antibody Tests with Conventional IIF Assay

Y. Kim, H. Mo, S. Yoon, Y. Kim, J. Choi, G. Oh. *Eone Laboratories, Incheon, Korea, Republic of*

Background: Antinuclear antibodies (ANA) are well-appreciated biomarkers in the laboratory diagnostics of systemic autoimmune rheumatic diseases. While indirect immunofluorescence (IIF) assays using human epithelial-2 (HEp-2) cells has been established as the gold standard method of detecting ANA, it is labor-intensive and subject to variation due to the handling practices of laboratory staff. To overcome these limitations, several automated systems for computer-aided IIF microscopy have recently been introduced. The aim of this study was to investigate the reliability of two automated IIF systems for ANA tests (NOVA View, INOVA Diagnostics, USA and EUROPattern Suite, Euroimmun AG, Germany).

Methods: A total of 200 residual serum samples, including 100 ANA-positive and 100 ANA-negative sera were collected. We tested ANA using two automated IIF systems with default settings and compared with the results obtained by the conventional IIF assay (ZEUS IFA ANA HEp-2 Test System, ZEUS Scientific, Inc. USA). After adjusting the software setting, additional 78 samples were compared. The ability of ANA detection and pattern recognition for five basic patterns (homogeneous, speckled, nucleolar, nuclear dots and centromere) was evaluated.

Results: The overall agreements of both automated IIF systems with the conventional IIF assay were 72% (Kappa 0.44) by NOVA View and 57% (Kappa 0.13) by EUROPattern Suite. Though positive agreements were excellent (93% for NOVA View and 99% for EUROPattern Suite), both systems showed poor negative agreements (51% and 14%, respectively). The agreements were improved (NOVA View: 76%, Kappa 0.68 and EUROPattern Suite: 82%, Kappa 0.74) after software adjustment because false positive results were reduced. Of 105 positive results by conventional

IIF assay, NOVA View correctly identified 52/105 (59%) ANA patterns; the recognition accuracies of the homogeneous, speckled, nucleolar, nuclear dots and centromere were 93%, 31%, 35%, 33% and 100%, respectively. EUROPattern Suites correctly assigned 69/105 (68%) ANA patterns; the recognition accuracies of the homogeneous, speckled, nucleolar, nuclear dots and centromere were 100%, 63%, 35%, 44% and 100%, respectively.

Conclusion: In this study, the ANA detection capability of two automated IIF systems showed good agreement with the conventional IIF assay. The ability of the pattern recognition showed various degrees depending on the pattern. Since, the most important features of automated IIF systems are reliable identification for negative results, it is necessary to adjust the software for each laboratory. Also, it would be helpful to visually confirm the automatic results in terms of accurately reporting the ANA pattern.

A-103

Comparison of Manual and Automated Flowcytometry Gating Tools for Lymphocyte Subset Analysis in Hematopoietic Stem Cell Transplantation Recipients

Y. Kim[1], H. Lee[2], J. Ryu[3], E. Oh[3]. *[1]Department of Laboratory Medicine, EONE Laboratories, Incheon, Korea, Republic of, [2]Department of Laboratory Medicine, International St. Mary's Hospital, College of Medicine, Catholic Kwandong University, Incheon, Korea, Republic of, [3]Department of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of*

Background: Automated gating analysis of lymphocyte subset has been widely used to decrease the individual source of variation and to save analysis time in clinical laboratories. However, on the challenge of using an automated analysis in hematopoietic stem cell transplantation (HSCT) recipients, a variety of lymphocyte subset results is expected according to the transplantation time and recovery ability of individual patients. In this study, we evaluate the applicability of the automated gating program in HSCT by comparing the results between automated gating and conventional manual gating.

Methods: In 22 HSCT recipients, lymphocyte subset analyses were performed using Beckman Coulter Navios (Beckman Coulter, USA) with Navios Tetra software. The lymphocyte populations (CD3+ T cells, CD4+/CD8− T cells, CD4−/CD8+ T cells, CD3−/CD19+ B cells and NK cells) were enumerated. The reagent for Navios consisted with two tubes: one for CD45-FITC, CD4-RD1, CD8-ECD, CD3-PC5 and the other for CD45-FITC, CD56-RD1, CD19-ECD, CD3-PC5 (CYTO-STAT tetra CHROME, Beckman Coulter, USA). Gating was done both manually by experienced lab technicians and verified by a medical supervisor and, with automated programs. The daily quality control measures include analysis of Immunotrol at both normal and low levels (Beckman Coulter).

Results: For Immunotrol normal cells, CVs for CD3+, CD4+, CD8+ T cells were less than 5% and less than 10% for NK and B cells with no significant difference between manual and automated gating. For Immunotrol low cells, all CVs were less than 10% except for NK cells with automated gating. On Navios flowcytometer, CD8+ T cell results using automated gating were consistently higher by 3-4% than results using manual gating. Automated gating strategies affected CD8 results in four patients (18.2%). In addition, CD4+CD8+ dual positive population and CD56 dim+ NKT cell population were not adequately analyzed in automated gating analysis. Automated gating excluded lymphocytes with low FSC and high SSC. It is also necessary to add CD16 reagent in Navios system because HSCT recipients had high proportion of CD56-CD16+ NK cells in lymphocyte population and it cause misinterpretation of data in automated gating strategy.

Conclusion: When performing automated gating analysis in HSCT recipients, it is necessary to confirm histogram and gating results.

A-104

Development of Non-Competitive Immunoassay of Aldosterone for LUMIPULSE® G Systems

T. Yoshimi, S. Kojima, K. Teruyama, H. Yui, T. Sakyu, T. Shirakawa, K. Moriyama, K. Aoyagi. *FUJIREBIO INC., Tokyo, Japan*

Background: Aldosterone, the primary mineralocorticoid, is a steroid hormone with molecular weight of 360 daltons and is produced by the zona glomerulosa of the adrenal cortex. The central role of aldosterone is in the homeostatic regulation of plasma sodium and potassium levels, therefore, regulating blood pressure. The plasma aldosterone concentration (PAC) assay is important in the diagnosis of primary hyperaldosteronism (PA) and renovascular hypertension, etc. For screening and diagnosis of such diseases, high quantitativeness is necessary. We have developed a novel, fully-automated, high-quantitative noncompetitive chemiluminescence immunoassay for LUMIPULSE G system for detecting Aldosterone in serum and plasma, and its performance is evaluated.

Methods: Aldosterone assay for LUMIPULSE system is a two-step sandwich chemiluminescent enzyme immunoassay (CLEIA) without a specific pretreatment process prior to the 1st immunoreaction. The resulting reaction signals are derived within 30 minutes/sample, and are proportional to the amount of aldosterone in the sample allowing quantitative determination of aldosterone in serum and plasma.

Results: Limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were 0.9 pg/mL, 2.1 pg/mL and 7.9 pg/mL, respectively. A 20-day precision study was performed during a 31-day period using two controls and three panel specimens, and the imprecision was ≤ 3% total CV. Dilution linearity was evaluated using three test samples, and the recovery rate of up to 200-fold dilution was 95-109% for manual dilution within the calibration range of 20 - 2,000 pg/mL. For spike recovery study, varying amounts of aldosterone were added to serum and plasma samples containing low levels of aldosterone to create test samples with concentrations ranging from 432.2-1656.3 pg/mL. The measured values, when compared to the expected values, ranged from 98-102%. The correlation coefficient and the regression slope of this assay and LC-MS/MS method (Aska Pharma Medical Co., Ltd., Kawasaki, Japan) were 1.00 and 1.00, respectively (N=130). The measurement value variations by various interferences (bilirubin, hemoglobin, triglycerides, total protein, chyle, creatinine, rheumatoid factor, HAMA, etc.) and cross-reactivity (cortisol, corticosterone, 11-deoxycorticosterone, progesterone, estradiol, testosterone, etc.) were ≤ 10% at clinically sufficiently high concentrations.

Conclusion: Our novel aldosterone assays for LUMIPULSE G systems was well-correlated with LC-MS/MS method and also was able to accurately quantify low level samples. Aldosterone assay for LUMIPULSE may be useful for the routine analysis of PAC and for the screening and diagnosis of PA from large number of hypertensive patients.

A-105

Development of Novel Immunoassay for the Quantitation of Direct Renin using LUMIPULSE® G Systems

K. Hamano, H. Saito, S. Kojima, T. Yoshimi, K. Moriyama, K. Aoyagi. *Fujirebio Inc., Tokyo, Japan*

Background: Renin controls blood pressure via renin-angiotensin-aldosterone system. Measurement of the renin concentration is useful to screen primary aldosteronism from large number of hypertensive patients. There are two kinds of method to measure renin in plasma currently used in clinical settings, plasma renin activity (PRA) and plasma renin concentration (PRC). PRC assays have advantages in terms of rapidity and easy sample management. However, conventional PRC assays may have poor correlation with PRA under treatment with direct renin inhibitor such as aliskiren. Last year we reported the establishment of a unique anti-renin antibody which not reacts with inactivated renin under existence of aliskiren. Based on the unique characteristics of this antibody, we have developed the high sensitive chemiluminescence immunoassay for detecting direct renin using fully-automated LUMIPULSE systems, and its performance is evaluated.

Methods: Direct renin assay using LUMIPULSE systems is a two-step sandwich chemiluminescent enzyme immunoassay (CLEIA). The resulting reaction signals are derived within 30 minutes/sample, and are proportional to the amount of renin in the sample allowing quantitative determination of direct renin.

Results: The detection limit of the assay was 0.09 pg/mL, and the limit of quantitation was 0.20 pg/mL. A precision study was performed during a 32-day period using three controls and three panel specimens, and the imprecision was ≤ 3% total CV. Dilution linearity was evaluated using three test samples, and the recovery rate of up to 100-fold dilution was 98-108% for manual dilution within the calibration range of 0.02 - 1,000 pg/mL. For spike recovery study, varying amounts of renin were added to plasma samples containing low levels of renin to create test samples with concentrations ranging from 278.37-947.76 pg/mL. The measured values, when compared to the expected values, ranged from 94-99%. The correlation coefficient and the regression slope of this assay and conventional IRMA method (Renin IRMA "FR", Fujirebio Inc., Tokyo, Japan) were 0.96 and 1.02, respectively (N=34). Moreover the direct renin concentrations in samples which were treated with aliskiren is well correlated to PRA as same as non-treated samples.

Conclusion: Our novel direct renin assay using LUMIPULSE G systems is observed good correlation with PRA even in the treatment of ariskiren. In addition, this new automated PRC assay can reduce dramatically Turn Around Time compared to PRA assay because of unnecessity of Angiotensin I production by enzymatic reaction. This

assay is considered as useful for the routine analysis of PRC and the diagnosis for primary aldosteronism from large number of hypertensive patients.

A-106

Performance of a Low Level Total Protein Assay for Use on the Binding Site Optilite Analyser

R. J. van Heezik, P. J. Showell, J. Griffiths, R. Lyon, M. McCusker, S. J. Harding. *The Binding Site Ltd, Birmingham, United Kingdom*

Measurement of total protein in urine is a useful marker for disorders that cause increased glomerular filtration or decreased protein reabsorption in the kidney. Increased levels in cerebrospinal fluid (CSF) can indicate increased permeability of the blood/brain barrier or synthesis of immunoglobulins in the central nervous system. Here we describe the evaluation of a Low Level Total Protein assay for use on the Binding Site's Optilite® analyser. The instrument is a random-access turbidimetric analyser, capable of on-board sample dilutions up to 1/10,000 and throughput of up to 120 tests per hour. Precision is promoted by single-use cuvettes, which are automatically loaded and disposed of. The assay is programmed to produce a single point calibration curve, with a measuring range of 40-1100mg/L and sensitivity of 20mg/L using neat samples. High samples are automatically re-measured at a dilution of 1/11, with an upper limit of 6000mg/L. A linearity study was performed (EP06-A) using both urine and CSF samples spiked with normal human serum. The assay was demonstrated to be linear over a range of 36mg/L - 1210mg/L. Interference testing was performed (EP07-A2) at a single urine and CSF concentration, challenging with ascorbic acid (CSF) and creatinine, urobilinogen and conjugated bilirubin (urine). All results were <±10% when compared to equivalent negative controls. A precision study was performed (EP05-A2), by testing 3 urine levels and 2 CSF levels on a single analyser over 20 days:

	Precision (%CV)			
Precision Level	**Within-run**	**Between-run**	**Between-day**	**Total**
Urine 1 – 60mg/L	5.4	7.1	4.2	9.9
Urine 2 – 140mg/L	3.7	3.3	0.0	5.0
Urine 3 – 1070mg/L	0.4	3.6	0.4	1.5
CSF 1 – 450mg/L	0.6	3.6	2.3	4.3
CSF 2 – 1070mg/L	0.6	1.4	0.6	1.7

Comparison was made to the Siemens ADVIA 1800 Total Protein 2 assay, using clinical samples (n=80, range 25 - 5201mg/L). Good agreement was observed when the data was analysed by Passing-Bablok regression; y=1.06x - 25.8. In conclusion, the Low Level Total Protein assay for use on the Optilite® provides a reliable and precise method for quantifying total protein in urine and CSF samples and correlates well with existing methods.

A-107

Performance Comparaison of the Atellica, and Alinity I, Hepatitisand Retrovirus Assays in Routine Laboratory Testing

J. Ducos, J. Guiraud. *CHU Montpellier, Montpellier, France*

Background: The Hepatitis A, B, C and HIV immunoassays are critical serological tests in the diagnosis and evaluation of organ donations and blood screening in our laboratory in CHU Montpellier, France. Our Laboratory decided to evaluate two systems from different vendors for their performance on these assays. The aim of the study was to evaluate the precision, specificity and sensitivity of these assays on the two systems.

Methods: The Atellica assays (HBsAg, HBs Ag Quant, HBe, HBs Ab, HAVAb G, HAVAb M, HB core, HB Core M, HCV, HIV) were tested side by side with the corresponding Alinity i assays. Precision (repeatability, and reproducibility) was determined using pooled patient samples. Clinical specificity was assessed using selected blood and routine diagnostic specimens, clinical sensitivity was determined using confirmed positive specimens. Specimens where confirmed via PCR or Western blot depending on the disease state and the method available. Carryover from a high positive to negative samples was evaluated for the HBsAg and HIV assays on the two systems. Correlation for the Quantitative HBsAg assays was done utilizing dilutions of High positives to generate values across the dynamic range of the assays.

Results: The repeatability of the HAVAb G, HAVAb M assays ranged between 3.2% and 14.4% CV depending on the sample S/CO level tested and the system used. The Hepatitis B envelope and core assays ranged in precision from 1.8% to 7.3% depending on level of panel and the assay being tested. The repeatability of the HBsAg Quantitative assays ranged between 6.6% and 11.1% CV and these assays had a correlation coefficient R = 0.974. On the other hand, the HBs Antibody assays correlation with an R value of 0.872 and a slope of 0.83. The HIV and HCV assays demonstrated precision values of 2.1% to 6% CV had a concordance of 90.85% and 95.5% (n = 153 and 131) respectively with most of discordant values coming mainly from false positives.

Conclusion: The performance characteristics of the Atellica and Alinity assays used for routine Hepatitis and HIV testing while comparable point to critical differences in false positive rates (specificity). Mitigating false positives is critical to our organ program, to ensure patients in need receive lifesaving and timely transplants.

A-108

Comparison of Complement C2 Assay Performance on the Binding Site Optilite® Turbidimetric Analyser Using Different Calibration Methods

D. McEntee, C. Evans, D. Matters, M. McCusker, S. Harding. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Background:

An assay has previously been developed for a C2 assay for the Binding Site SPAPLUS® analyser; here we describe the performance of two potential C2 assay formats for the Binding Site Optilite analyser. One format is based on the use of a multi-calibrator set as used on the SPAPLUS and the second utilises a single calibrator and the analyser's capability to perform calibrator dilutions in order to produce the calibration curve. C2 is a β1-glycoprotein which forms part of the classical complement pathway. It is cleaved by activated C1s into two fragments, C2a and C2b. The larger fragment of C2 then combines with C4b to produce C3 or C5 convertase. Reduced C2 plasma concentrations result from classical complement pathway activation, i.e. from immune complex mediated activation. C2 deficiency is the most common inherited complement component deficiency and is associated with systemic lupus erythematosis, glomerulonephritis and vasculitis. The measuring range of both Optilite assays is 4-45mg/L at the standard dilution of 1+9; the overall range extends up to 90mg/L via auto repeat to 1+19 for high samples.

Methods: A precision study was performed according to CLSI EP5-A3 over 5 days using one reagent lot on one Optilite analyser. The study was carried out using 3 samples with different analyte concentrations. The acceptance criteria was <8% CV for total precision. A comparison study to the Binding Site SPAPLUS C2 assay was performed using 37 serum samples ranging from 17.92mg/L to 59.39mg/L and 38 EDTA plasma samples ranging from 15.99mg/L to 67.06mg/L. Results were analysed for concordance between platforms based on a cut off value of 18.934mg/L. A linearity study was completed following CLSI EP06-A.

Results: The total precision coefficients of variation (CVs) were as follows: 4.9% at 9.68mg/L, 3.9% at 21.25mg/L and 3.2% at 33.85mg/L for the multi-calibrator assay and 5.5% at 8.67mg/L, 3.6% at 19.75mg/L and 3.1% at 31.61mg/L for the single calibrator assay. The comparison yielded the following results: 100% agreement between the SPAPLUS and both the multi calibrator and single calibrator Optilite assays based on serum samples; 100% agreement between the SPAPLUS and the single calibrator Optilite assay and 97.1% agreement between the SPAPLUS and the multi calibrator Optilite assay based on EDTA plasma samples. The linearity demonstrated a linear response over the range from 0.6mg/L to 4.2mg/L for both the multi-calibrator and the single calibrator assays on the Optilite at the neat analyser dilution. This is equivalent to a range of 6mg/L to 42mg/L at the standard 1+9 dilution.

Conclusion: Both the multi calibrator and the single calibrator C2 assays for the Optilite yield reliable and precise data for quantifying C2 concentrations.

A-109

Evaluation of Anti-Nuclear Antibody Test Utilization Patterns in Alberta

A. C. Chin[1], J. Viczko[2], J. W. Cohen Tervaert[3], A. Russell[3], R. Ferrari[3], J. E. Raizman[4], B. Kyle[5], A. E. Clarke[6], M. J. Fritzler[6], C. Prosser[4]. [1]*Alberta Public Laboratories and Department of Pathology and Laboratory Medicine, University of Calgary Cumming School of Medicine, Calgary, AB, Canada,* [2]*Alberta Public Laboratories, Calgary, AB, Canada,* [3]*Department of Medicine, Division of Rheumatology, University of Alberta Faculty of Medicine and Dentistry, Edmonton, AB, Canada,* [4]*Alberta Public Laboratories and Department of Laboratory Medicine and Pathology, University of Alberta Faculty of Medicine and Dentistry, Edmonton, AB, Canada,* [5]*DynaLife Medical Labs, Edmonton, AB, Canada,* [6]*Department of Medicine, Division of Rheumatology, University of Calgary Cumming School of Medicine, Calgary, AB, Canada*

Background: Antinuclear antibody tests (ANA) are ordered on patients suspected of having an autoimmune disease (AD). The utilization of ANA screening restricted to patients with high pretest probability of an AD could potentially reduce the volume of orders, increase positive testing rates and subsequent appropriate consultations by tertiary care physicians. However, restricting orders could also lead to missed or delayed diagnoses of evolving AD. As an approach to develop more effective strategies and policies, we assessed ANA utilization patterns in Alberta.

Methods: ANAs were performed in five laboratories employing automated computer-aided or manual indirect immunofluorescence assays (IFA), or a solid phase addressable laser bead immunoassay. Data was retrospectively obtained from three laboratory information systems.

Results: Approximately, 1/40 Albertans had an ANA done at annual costs approaching $900,000. This rate is inconsistent with the frequency of new cases of systemic autoimmune rheumatic diseases in Alberta (2.6 per 1000). Most ANA were ordered by primary care physicians (76%) while only 6% were ordered by rheumatologists and 25% by other specialists, which indicates the increasingly widespread use of ANA. The increase in ANA positivity rate may be partly attributed to the gradual expansion of IFA pattern interpretation as recommended by the International Consensus of ANA Patterns at one laboratory. In addition, 6% of patients had a repeat ANA within 6 months, but it is not known if there was a strong suspicion of an evolving pathology or change in the patient's illness at the time.

Conclusion: There appears to be overutilization of ANA in Alberta which may lead to unnecessary expenditures and referrals. Other jurisdictions have previously implemented ANA testing recommendations. These findings will lead to the implementation of specific ordering indications for ANA, while observing the downstream consequences of costs and impact on detection of AD.

A-110

Evaluation of Variability and Combinability of Fecal Calprotectin (FCP) Results Based on the Extraction Process of Calprotectin from Stool Samples

S. Narla[1], M. Hu[1], J. Robinson[1], I. Patzer[1], J. Sims[1], M. Florent[2]. [1]*Covance Central Laboratories, Indianapolis, IN,* [2]*Covance Central Laboratories, Geneva, Switzerland*

Background: Calprotectin is a calcium-heterodimer protein which is abundant in the cytoplasm of neutrophils. This is a biomarker with good sensitivity and specificity in case of inflammatory Bowel disease (IBD) which is a chronic inflammatory gut. In case of IBD, neutrophils from the inflammatory area release calprotectin, which leads to its increased levels in stool samples. Calprotectin is measured in extracted stools. There are several extraction devices that are commercially available as well a manual weigh-in method, "Gold Standard" method. The homogeneity of stool sample and the neutrophil levels in the sample affect the precision of the results from the same stool sample. Our study compares 2 commercial stool extraction devices with the manual weigh-in method as well as the variability within each extraction method.

Methods: Twenty five unique stool samples were extracted in triplicate using three different techniques: manual weigh-in, Smart prep extraction device and CALEX CAP extraction device following the manufacturer's directions. All the samples were tested in parallel using a BÜhlmann ELISA kit that was validated as per CLSI guidelines in the laboratory. The intra-extraction precision and the bias between extraction methods were evaluated. The manual extraction is considered as "Gold standard" for evaluation of both commercial extraction devises.

Results:

Statistical Parameter	Method	Result
% CV Range, %CV Mean	Manual Weigh-in	0.8 to 27.9%, 14.3 %
	Smart Prep	2.8 to 48.6%, 17.3 %
	CALEX Cal	3.1 to 50.9%, 12.6%
% Bias Range % Bias Mean	Smart Prep vs. Manual	3% to -80 %, -37%
	CALEX Vs. Manual	0% to -79%, -13 %
Regression Analysis	Smart Prep vs. Manual	Y= 0.747X - 68.57, X= Manual method, Y= Smart prep
	CALEX Vs. Manual	Y = 0.890X- 40.41, X= Manual method, Y= CALEX

Conclusion: All three methods displayed a wide range of variability within each extraction process. The commercial extraction devices displayed a bias up to -80% (Smart Prep) and -79% (CALEX) compared to the results from manual extraction. However the CALEX extraction displayed less overall bias than the Smart prep in correlation with manual weigh-in. Laboratories should be aware of the imprecision between each extraction process and should follow one method of extraction for data consistency, and the data from the different extraction methods should not be used interchangeably.

A-111

Serums Lactate Dehydrogenase Levels Correlated with Patients' Paroxysmal Nocturnal Hemoglobinuria Clone Sizes

W. Wang[1], H. Li[2], Y. Wang[2], G. Fan[2], L. Shen[1]. [1]*Xinhua hospital, Shanghai Jiaotong University of Medicine School, Shanghai, China,* [2]*Oregon Healthy and Science University, Portland, OR*

Background: Paroxysmal nocturnal hemoglobinuria (PNH) often presents as hemolysis and/or bone marrow failure. Flow cytometry testing can accurately detect PNH. However, it's not clear if PNH clone size correlates with severity of hemolysis using lactate dehydrogenase (LDH) as an indicator. So, we reviewed the positive PNH patients' clinical history to investigate if the PNH clones correlate with LDH levels.

Methods: High sensitivity PNH flow cytometry (0.01% limit of detection) was performed with FLAER-FITC, CD64-PE, CD14-ECD, CD15-PC5, CD24-AA7500, CD45-KO for neutrophils (PMN) and monocytes (Mono); CD59-PE and CD235a-AA750 for RBCs. Retrospective analysis was done in the positive PNH cases from 2013-2019 at our hospital. Total 173 cases for 57 patients, 27 females and 30 males, 52 adults and 5 pediatrics; with an age range 9-78.

Results: Among 57 patients, there are 30 aplastic anemia (AA), 7 AA progressing to PNH (AA/PNH), 5 myelodysplastic syndromes (MDS), 12 PNH patients, 1 pancytopenia, 1 autoimmune disease and 1 thrombosis. The mean and range of PNH clone and LDH concentrations for patients are listed in Table. Significantly higher levels of all PNH clones were observed in PNH and AA/ PNH, compared to AA (all $P < 0.001$) and MDS (all $P < 0.05$).LDH was higher in PNH and AA/PNH than AA and MDS groups ($P < 0.001$). LDH demonstrated positive correlation with PNH clone size in RBC-type-III, neutrophils and monocytes (all $P < 0.0001$, R= 0.4447, 0.5469, 0.5711, respectively).No correlation was observed between LDH and RBC-type-II.

Conclusion: Positive PNH was most frequently seen in AA patients. AA has lower PNH clones and LDH than those of PNH patients or AA/PNH group. For all patients, LDH concentrations showed positive correlation with PNH population. In addition, normal LDH level can not rule out possibility of positive PNH. For patients with normal LDH level and persistent pancytopenia, PNH flow cytometry testing is warranted.

Table. Clinical characteristics of PNH tesing positive patients.

Diseases	AA	AA/PNH	MDS	PNH
Number	30	7	5	12
Percent (%)	53.63	12.28	8.77	21.05
RBC-II (%)	0.06(0.00-0.50)	1.60(0.01- 33.22)	0.04(0.01-0.10)	3.70(0.01-21.99)
RBC-III (%)	0.38(0.00-9.17)	9.21(0.41-50.15)	0.22(0.00-0.82)	32.63(0.75-100)
PMN (%)	3.01(0.01-15.43)	28.06(0.92-98.94)	4.79(0.04-17.09)	84.22(16.36-99.05)
Mono (%)	2.80(0.01-14.50)	27.03(1.50-99.07)	3.83(0.00-10.80)	86.08(4.40-99.89)
LDH(≤ 250U/L)	212.76(109-610)	397.09(150-2120)	239.75(164-340)	1164.83(142-4875)

A-112

Evaluation of the Potential Anti-Inflammatory Effect of Semi-Synthetic Anacardic Acid (LDT13) Extracted from Cashew Nut Shell Liquid (CNSL)

M. Q. Souza, I. M. S. N. Teotônio, G. S. Heyn, P. S. Alves, L. S. Romeiro, R. Pratesi, Y. M. Nóbrega. *UNIVERSITY OF BRASILIA, Brasilia, Brazil*

Background: Inflammation is a complex, highly regulated biological process defined as the organism defense response to any aggressive agent. The inflammatory process aims to the recovery of the damage tissue homeostasis. However, a chronic inflammatory response may progressively lead to the loss of organ or tissue function resulting in disease development. In the cashew nut processing industry, the cashew nuts shell produces an oil that is traditionally known as cashew nut shell liquid (CNSL). Studies have shown that the CNSL derivatives, mainly the anacardic acid, have antioxidant and anti-inflammatory activity. However, the use of this by-product of the cashew nut industry is practically neglected in Brazil. The multi-target pharmacological profile of anacardic acid found in CNSL makes it a potential target for the development of novel anti-inflammatory agents. The present work proposes to evaluate the antiinflammatory effect of a semisynthetic derivative extracted from phenolic lipids of Anacardium occidentale L., denominated LDT13.

Methods: Organic synthesis of the phenolic derivative of CNSL giving the hemi-synthetic compound LDT13. The cytotoxicity of the proposed compound, LDT13, was analyzed in murine macrophages cell line, RAW264.7. The cells were previously stimulated with lipopolysaccharide (LPS) inducing inflammation. Then were treatment with LDT13, in times of 6h and 24h. The analysis of the gene expression of inflammatory markers (*iNOS, NF-κB, IL-1β* and *IL-6*) was performed by real-time PCR method, nitric oxide (NO) dosage by spectrometry, and cytokine IL-6 by the ELISA technique. **Results:** The results showed that LDT13 influenced the gene modulation of inflammatory mediators. The relative quantification of the gene transcripts showed that the proposed phenolic derivative disclosed a potential anti-inflammatory effect evinced by the decrease of the markers gene expression when compared to control group composed by cells stimulated with LPS without further treatment with LDT13. After a 6-hours treatment an average reduction of 55.7% was observed of the inflammatory markers expression. After 24-hour treatment the mean reduction in gene expression was 84.5%. The NO and IL-6 assays confirmed the results obtained in gene expression. The NO reduction was, on the average, 94.5% and the IL-6 dosage reduction an average of 92.8%. **Conclusion:** LDT13 was shown to be a potential anti-inflammatory agent, having a rapid effect. Treatment with LDT13 decreases inflammatory marker gene expression, NO and IL-6 production. The anti-inflammatory activity of LDT13 was shown to be superior compared to commercial drugs tested.

A-113

Comparison of aAutomated Fluorescence Enzyme Immunoassay (FEIA) with Microplate ELISA for Identification of Anti-Extractable Nuclear Antigens (anti-ENA) in Clinical Samples

J. Oh, K. Lee, H. Kim. *Yonsei University College of Medicine, Seoul, Korea, Republic of*

Background: There is no gold standard method for detecting autoantibodies to extractable nuclear antigens (ENAs) yet. In the aspect of finding a better assay which could provide more satisfactory diagnostic performance, we compared two current assays commonly used. An automated fluorescent enzyme immunoassay, Phadia® 250, was compared to microplate ELISA assay for the detection of five common anti-ENAs.

Methods: A total of 74 sera from the patients with systemic autoimmune diseases and 26 healthy controls were collected and assayed with the Phadia® 250 (Phadia, Sweden) and microplate ELISA (INOVA Diagnostics, USA) for 5 anti-ENAs (anti-SS-A/Ro, anti-SS-B/La, anti-RNP, anti-Sm and anti-Scl-70). The statistical analyses of agreement rate with kappa coefficient, sensitivity and specificity of each assay were performed.

Results: The concordance rates between ELISA and Phadia® 250 ranged from 89% for anti-RNP to 97% for anti-Scl-70, and the kappa coefficients between the results by the two assays were from 0.44 to 0.82. There were no significant differences in sensitivities and specificities of each anti-ENA between the two assays except for specificity of anti-RNP and sensitivity of anti-Sm.

Conclusion: In this study, Phadia® 250 and microplate ELISA showed comparable results for detecting autoantibodies in clinical specimens. Automated FEIA by Phadia® 250 could be a useful method in that it provides shorter protocol time, full automated and standardized methodology.

A-114

Utility of Mouse-Human Chimeric IgE Antibodies with Defined Allergen Monospecificities in Determining the Accuracy of Heterologous Interpolation Methods Used in Commercially Available Allergy Diagnostic Platforms

E. Sinson, R. Asprer, S. Nguyen, C. Ocampo. *Hycor Biomedical, Garden Grove, CA*

Background: Laboratory tests for allergen-specific IgE (sIgE) antibodies are valuable in identifying individuals who are sensitized to one or many allergens and thus at risk for allergic symptoms following a subsequent allergen exposure. However, some studies have noted large discrepancies among results obtained by different manufacturers. Such discrepancies have been mainly attributed to variability in the allergen raw material used but not many studies have been done to evaluate the accuracy of each assay's calibration system in contributing to the discrepant results. Currently, there is no universally accepted IgE antibody standards with calibrated levels of allergen-specific IgE and thus require a heterologous interpolation of sIgE results from a total IgE (tIgE) dose response curve. It is a cause of concern that using different methods to establish a reference curve and measure sIgE in samples may generate inaccurate results.

With the increasing availability of mouse-human chimeric IgE antibodies with known monospecificities, heterologous calibration systems can now be evaluated for accuracy. As an example, this study evaluated the accuracy of allergen Fel d 1 using an in-house validated assay, Phadia™ 1000 System, and IMMULITE® 2000 Instrument using a chimeric IgE antibody.

Methods: Mouse-human chimeric IgE antibody monospecific for allergen component Fel d 1 was diluted in IgE-free stripped serum and titrated to two levels. The diluted samples were then tested on the in-house assay, Phadia 1000, and IMMULITE 2000 and compared against their total IgE and specific IgE (Fel d 1) result. In this approach, the chimeric IgE comprises the entire amount of IgE present in the sample, allowing assessment of the system's calibration of sIgE by comparing the sIgE and tIgE measurement it delivers.

Results: The chimeric antibody levels gave an equivalent response in the total IgE and specific IgE with an average ratio of 0.82 on the in-house assay and an equivalent average ratio of 0.74 on Phadia 1000. However, IMMULITE 2000 resulted in an average ratio of 3.44 signifying a substantially higher sIgE result compared to its total IgE value.

Conclusion: Although the in-house assay and Phadia 1000 system demonstrates comparable sIgE / tIgE ratios, IMMULITE 2000 reported sIgE concentrations to Fel d 1 were on average 3-fold higher than the tIgE concentrations in the same sample.

These findings demonstrate that IMMULITE 2000 delivers overestimated sIgE levels compared to the other platforms tested in this study. We conclude that systematic differences in the calibration system can contribute to discrepant results among different manufacturers and the utilization of mouse-human chimeric IgE antibodies can be an effective tool in assessing the accuracy of allergy platforms.

A-115

Assessment of ANA HEp-2 IFA Result Interpretation Using the dIFine Imaging System

D. Ryan, K. Cichonski, J. Torres, M. Kopnitsky, D. Zweitzig. *ZEUS Scientific, Branchburg, NJ*

Background: Indirect fluorescent antibody (IFA) methods utilizing the HEp-2 cell line have long been utilized for detecting anti-nuclear antibodies (ANA) from the sera of patients with systemic lupus erythematosus, and other autoimmune disorders. For decades, ZEUS Scientific (ZS) has manufactured FDA-cleared IFA ANA HEp-2 Test Systems that rely upon manual interpretation of results using a fluorescent microscope. This HEp-2 IFA Test System was recently utilized to develop software algorithms in conjunction with a specialized microscope, towards interpretation of qualitative and ANA pattern results in an automated fashion ('ZEUS dIFine' system). The objective of this study was to assess the performance of the newly developed dIFine system, relative to manual IFA interpretation, using pre-characterized serum samples.

Methods: Three independent HEp-2 IFA positive patient sera were selected for each of following ANA patterns [Homogenous, Centromere, Speckled, Nucleolar, Anti-mitochondrial antibody (AMA), Ribosomal, Nuclear Membrane (NM), and Nuclear Dots (ND)], as well as 50 negative samples. Each of the 24 ANA pattern-positive samples were serially tittered and assayed by the HEp-2 IFA Test System. Negative samples were assayed only at a 1:40 screening dilution. The endpoint titer for each pattern-positive sample was determined manually, then the same slides were scanned using the dIFine system; towards generating parallel automated determination of endpoint titer and pattern recognition for each slide well. For the 24 positive samples, agreement between manual and dIFine interpretations was determined for ANA pattern results, as well as endpoint titer values. For negative samples, only qualitative agreement was evaluated.

Results: Endpoint titers for each of the 24 pattern-positive samples were in excellent agreement between the manual and dIFine interpretations (i.e. last positive result obtained for each sample was within +/- 1 dilution between each interpretation method). Manual and dIFine pattern determinations were in 100% agreement for each of the 24 pattern-positive samples, at all dilutions yielding a positive qualitative result. 100% (50/50) of the negative samples were also determined to be negative by the dIFine instrument.

Conclusion: This study represents the first the qualitative result interpretation and ANA pattern-recognition assessment for the newly developed dIFine imaging system. Additional analytical performance and clinical studies are being carried out, with the goal of further validating dIFine's qualitative result determination and pattern-recognition capabilities.

A-116

Contribution of Autoantibody Testing in the Evaluation of Patients At-Risk of Interstitial Lung Disease

A. S. Maharjan, A. E. Tebo. *University of Utah, Salt Lake City, UT*

Background: Interstitial lung disease (ILD) is a heterogeneous group of pulmonary conditions characterized by inflammation and fibrosis with diverse etiologies implicated in its pathology. It is a major complication of connective tissue disease (CTD) with variable occurrence, dynamics, and severity based on the specific type of CTD. A subset of patients referred to as interstitial pneumonia with autoimmune features (IPAF) may present with autoimmune symptoms but do not fulfil criteria for a defined CTD. In 2016, our institution initiated a comprehensive ILD autoantibody panel (ANA, RF, CCP, Jo-1, PL-7, PL-12, EJ, OJ, SRP, MDA5, NXP2, Ku, PM/Scl-100, U1RNP, and Scl-70) to assist in the diagnosis of CTD associated ILD (CTD-ILD). In this study, we investigated the contribution of a comprehensive autoantibody panel in the diagnosis of patients at risk for ILD.

Methods: Medical charts for consecutive patients that had testing for the ILD comprehensive autoantibody panel performed at ARUP Laboratories from the University of Utah Clinics were retrospectively reviewed for pulmonologist and rheumatologist notes. The presence or absence of ILD was determined for each patient. In addition, patients with ILD were categorized based on specific CTD, the frequency and characteristics of the different autoantibodies tested as well as other etiologies for ILD. Lastly, we compared the outcome for a diagnosis of ILD when the panel was positive versus negative (descriptive statistics). The University of Utah institutional review board (IRB) approved the study.

Results: Three hundred and three (n=303) patients received comprehensive ILD autoantibody panel testing during the study period. Of these, 59.7% (181/303) had a diagnosis ILD of diverse etiologies, 37.6% (114/303) non-ILD and 2.6% (8/303) undefined cases for ILD. CTD-ILD or IPAF was present in 17.1% (31/181) of the ILD cases. The CTD-ILD and IPAF cases included with rheumatoid arthritis 25.8% (8/31), IPAF 22.6% (7/31), anti-synthetase syndrome 16.1% (5/31), systemic sclerosis 12.9% (4/31), Sjogren's syndrome 12.9% (4/31), disease overlap 9.7% (3/31) and mixed connective tissue disease 3.2% (1/31). The CTD-ILD patients were mostly female 77.4% (24/31) and the mean age of the cohort was 64.3 (range: 48-84) years. ANA, RF, CCP and SSA-52 antibodies were the most common autoantibodies. In patients with anti-synthetase syndrome, Jo-1, PL-7 and EJ antibodies were the only markers positive. Overall, 10.9% (33/303) of patients with at least one positive autoantibody test identified in this panel had a diagnosis of CTD or ILD-CTD (including IPAF).

Conclusion: In this single center study, positive autoantibodies identified in the comprehensive ILD antibody panel significantly contributed to the diagnosis of CTD-ILD. Reliable and cost-effective biomarkers to identify patients at-risk for ILD irrespective of the underlying etiology would markedly improve early diagnosis.

A-117

Going Gel Free: Adding M-protein Quantitation to MASS-FIX in the Clinical Lab

K. A. Turner, S. Dasari, M. A. V. Willrich, D. L. Murray. *Mayo Clinic, Rochester, MN*

Background: The detection and quantitation of monoclonal proteins (M-proteins) are necessary for the diagnosis and monitoring of plasma cell dyscrasias. Despite limitations in gold-standard electrophoretic methods, protein gel electrophoresis (SPEP) and immunofixation electrophoresis (IFE) remain front-line tests. In July 2018, Mayo Clinic (Rochester, MN) implemented a novel method for the detection and isotyping of M-proteins utilizing immunoglobulin enrichment-coupled with matrix-assisted laser desorption ionization time-of-flight mass-spectrometry (MASS-FIX) as a replacement for gel-based IFE. With enhanced detection limits, the MASS-FIX is an automated and high through-put method capable of qualitatively detecting and isotyping M-proteins. Here we aim to validate MASS-FIX as a quantitative test by performing a method comparison between the MASS-FIX and SPEP M-protein quantitation. **Methods:** Retrospective clinical data was reviewed to obtain patient cases that had M-protein isotyping by MASS-FIX, SPEP quantitation (agarose gel electrophoresis with baseline gating, Helena Laboratories, Beaumont, Tx), and quantitation of the involved M-protein immunoglobulin performed simultaneously. The M-protein concentration was calculated from the MASS-FIX spectra utilizing the fractional area under the curve (AUC) of the M-protein which was then multiplied by the M-protein immunoglobulin concentration as determined by a nephelometric method (Siemens BNII, Marburg, Germany). The calculated fractional AUC was optimized through exploration of the type of integration (tangential skimming versus baseline) and peak width to give optimal agreement with M-protein concentrations obtained from SPEP. Using the optimal parameters, the method was applied to the additional patients from our clinical data set. **Results:** A cohort of 182 spectra from individual patients' spectra (consisting of 129 IgG, 19 IgM, and 34 IgM M-proteins) were analyzed and each isotype was correlated separately to its specific SPEP quantitation. Using a peak width of +/- 10 Daltons and dropping gates to the baseline produced fractional AUC which when multiplied by the immunoglobulin nephelometric quantitation (IgG, IgA, or IgM) produced M-protein concentrations in good agreement with SPEP. The correlation between the MASS-FIX and the SPEP for IgG was y=1.006x-0.003277, y=1.05x-0.1127 for IgA, and y=0.9027x+0.1074 for IgM. **Conclusion:** Here we demonstrate a correlation between the M-protein quantitation achieved by the novel mass spectrometry-based method MASS-FIX and the gold standard method of SPEP. Evidence suggests that validation of the qualitative MASS-FIX assay as a quantitative test to replace SPEP is possible. This method would substantially improve workflows in our laboratory and enhance turn-around time.

A-118

Performance Evaluation of ProteaseTag® Active Neutrophil Elastase Immunoassay in a Clinical Laboratory

L. Remigio[1], J. Busby[1], K. Moffitt[2], M. D. Galvan[1]. [1]*National Jewish Health, Denver, CO,* [2]*ProAxsis Ltd, Belfast, United Kingdom*

Background: Bronchiectasis is a chronic inflammatory condition characterized by significant neutrophil accumulation in the pulmonary alveolar parenchyma. Neutrophils release proteolytic enzymes, including neutrophil elastase, causing vascular injury in a variety of acute and chronic lung diseases. Recently, a prospective observational single center study demonstrated that elevated neutrophil elastase was associated with a higher frequency of exacerbations and lung function decline in bronchiectasis (1). These studies highlight the potential clinical diagnostic value of neutrophil elastase for bronchiectasis as well as other pulmonary diseases and therefore the need to evaluate the analytical performance of commercially available neutrophil elastase assays for clinical use.

Methods: Expectorated sputum was randomly collected from patients who had a chronic inflammatory lung disease and stored at -80°C until processing. The sputum was processed by a x5 dilution with phosphate buffered saline (PBS), mixed by inversion and centrifuged to extract the gel phase from the sol phase. Active neutrophil elastase in sputum sol was measured with the ProteaseTag® Active Neutrophil Elastase Immunoassay according to the manufacturer's (ProAxsis Ltd.) instructions. This assay employs ProteaseTag® Technology which allows the capture of active proteases such as neutrophil elastase in biological samples. Analytical performance of the assay was evaluated according to CLIA and CAP guidelines/specifications.

Results: Limit of the blank (LoB), and quantitation (LoQ) were 1.6 ng/mL, 4.2 ng/mL, respectively. Linearity was demonstrated over a range of 16 to 1000 ng/mL. The mean percent recovery over a dynamic range of neutrophil elastase concentrations was between 102.5% to 109.5%. Sputum sol samples were diluted x100 prior to testing to eliminate matrix effects and active neutrophil elastase was detected in 100% of the sputum sol samples tested, with a range of 1.6 mcg/mL to 19 mcg/mL. The % CV for intra-assay precision and inter-assay precision was ≤ 20%. Lastly, sample stability was determined as acceptable for up to two freeze/thaw cycles.

Conclusion: The ProteaseTag® Active Neutrophil Elastase Immunoassay demonstrated acceptable precision for detection of active neutrophil elastase in sputum sol samples. An extended study for clinical validation is planned and it is predicted that this assay could be useful as an aid in the diagnostic and risk management of patients with diseases such as COPD, cystic fibrosis, alpha-1 anti-trypsin deficiency and, bronchiectasis. (1) Chalmers, JD et al (2017)

A-119

Development of a High-Throughput Flow Cytometry Assay for Detection of Anti-MOG Antibodies

J. Fryer, S. Lopez-Chiriboga, J. Jitprapaikulsan, R. Sharma, A. Gadoth, E. Flanagan, S. Pittock, J. Mills. *Mayo Clinic, Rochester, MN*

Background: Antibodies targeting CNS-restricted membrane protein myelin oligodendrocyte glycoprotein (MOG) were first described in patients with multiple sclerosis (MS) utilizing non-conformationally- intact MOG antigen in either ELISA or Immunoblot formats. However, there was a high degree of discordance between different methodologies which confounded the relationship between MOG antibodies and multiple sclerosis. Specific assays emerged that utilized conformationally-intact cell surface MOG. The presence of MOG antibodies in these new assays associated with a disorder that was clinically, radiologically and pathologically different from MS. Despite the improvement in assay performance, early cell binding assays (CBA) methods lacked a specificity profile acceptable for clinical use. Flow cytometry-based assays utilizing cells expressing surface antigens as substrate have proven to provide a higher degree of specificity compared to traditional immunoassays. These flow cytometry-based approaches avoid target antigen distortion that plagues other assay formats and presents native conformation antigen. The study objective was to develop and optimize a flow cytometry-based method for the detection of MOG antibodies. **Methods:** HEK-293 cells were transiently transfected with a recombinant expression vector (pIRES2-AcGFP) that expresses human MOG and GFP from two independent ORFs. This cell population contains non-transfected cells (MOG^{neg}, GFP^{neg}) and transfected cells (MOG^{pos}, GFP^{pos}). Cells are mixed with 5 uL of patient serum diluted 1:20 with 2% BSA in PBS. Cells are then washed to remove unbound antibodies. Any remaining bound human antibodies are detected using an anti-human IgG1-Fc specific secondary antibody conjugated with AlexaFluor 647 (AF647). The cells are analyzed using a FACS Canto II. The amount of bound anti-MOG antibodies is calculated as the ratio of the median fluorescent intensity of AF647 of the MOG^{pos} GFP^{pos} population divided by that of the $MOG^{neg}GFP^{neg}$ population, termed the MOG IgG1 Binding Index (IBI). We assess intra and inter-assay imprecision, reference interval, LOB/LOD, analytical specificity, clinical sensitivity and specificity, accuracy (based on chart review and comparison with a commercially available CBA) and established optimal diagnostic IBI cut-offs. Samples with IBIs > 2.5 were diluted further to establish titers. **Results:** Assay imprecision - at an IBI near the cut-off of 2.5, the intra- and inter- assay CVs were <20% . Titer imprecision was assessed using 5 positive samples of varying end-point titers run over 20 assays. All positive samples fell within ± 1 dilution. The LOB for the IBI was 1.1 with a LOD of 1.9. Reference intervals - no healthy donors (50 adult, 50 pediatric) had detectable MOG antibodies (IBI <2.5). Analytical specificity -lipids, bilirubin nor hemoglobulin had no impact on IBI or end-point titer. Furthermore, MOG antibodies were not detected in 42 patients with hypergammaglobulinemia. Clinical sensitivity - 19 of 58 (33%) patients that fit clinical criteria (32 Acute disseminated encephalomyelitis, 26 AQP4 seronegative NMOSD) had IBIs >2.5. Clinical specificity- only 1 out of 224 MS patients (0.4%) had an IBI >2.5 . Compared to CBA, flow cytometry-based assays offered higher throughput, lower costs and superior specificity. **Conclusion:** We have developed a clinically sensitive and specific flow cytometry- based live cell assay that outperforms traditional slide-based CBA assays.

A-120

Outcome of a Large Multicenter Prospective Clinical Study Aimed at Assessing Agreement between Standard Two-Tiered and Modified Two Tiered Lyme Serology Testing Algorithms

D. Zweitzig[1], K. Cichonski[1], S. Ma[1], D. Ryan[1], J. Torres[1], D. Accardi[1], C. Keselica[1], M. Kopnitsky[1], E. S. Theel[2], D. Granger[2], J. Meece[3], T. Fritsche[3], J. Branda[4]. [1]*ZEUS Scientific, Branchburg, NJ,* [2]*Mayo Clinic, Rochester, MN,* [3]*Marshfield Clinic, Marshfield, WI,* [4]*Massachusetts General Hospital, Boston, MA*

Background: Since 1994, serology testing for Lyme disease in the United States has consisted of standard two-tiered testing (STTT) where specimens equivocal or positive by a screening methodology are further tested by IgG and/or IgM immunoblots. Several recent studies have recommended replacement of immunoblots with more sensitive and automatable second-tier tests, such as ELISA. This algorithm is termed modified two-tiered testing (MTTT). These studies have utilized banked samples, pre-segregated into Lyme disease and control groups. The goal of this study was to perform a multicenter prospective sample collection and assessment of agreement between STTT and MTTT algorithms.

Methods: Specimens submitted for routine Lyme testing were collected prospectively at three locations: Mayo Clinic (MOC), Rochester, MN.; Marshfield Clinic (MDC), Marshfield, WI; Massachusetts General Hospital (MGH), Boston, MA. All specimens were assayed by four ZEUS ELISA™ Test Systems: VlsE1/pepC10 IgG/IgM; *Borrelia burgdorferi* IgG/IgM; *Borrelia burgdorferi* IgG; and *Borrelia burgdorferi* IgM. ELISA testing of the MOC and MGH specimens was performed at each site, and the MDC specimens were tested at ZEUS Scientific [ZS]. Equivocal/positive specimens were assayed using IgG and IgM immunoblots at ZS (Trinity Biotech, Marblot™). Two-tiered results were characterized as follows: STTT [1st Tier - VlsE1/pepC10 IgG/IgM ELISA, 2nd Tier – IgG and IgM Immunoblot composite]; MTTT-A [1st Tier - VlsE1/pepC10 IgG/IgM ELISA, 2nd Tier - Borrelia burgdorferi IgG/IgM ELISA]; and MTTT-B [1st Tier - VlsE1/pepC10 IgG/IgM ELISA, 2nd Tier - Borrelia burgdorferi IgG and IgM ELISA composite]. Samples yielding discrepant STTT/MTTT results were assayed at ZS by a 'third-tier' ELISA (Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit). Positive percent agreement (PPA), negative percent agreement (NPA), total percent agreement (TPA), and kappa statistical analyses were performed for the MTTT algorithms relative to the STTT algorithm, with and without consideration of third-tier ELISA results.

Results: Data from 2,932 samples were available for STTT/MTTT agreement calculations [MOC (1042), MDC (990), MGH (900)]. Using STTT results as the comparator, agreement of the MTTT algorithms were: MTTT-A [PPA = 93.9% (CI = 90.3%-97.4%), NPA = 97.7 % (CI = 97.2%-98.3%), TPA = 97.5% (CI = 96.9%-98.1%); kappa = 0.81]; MTTT-B [PPA = 98.3% (CI = 96.5%-100.2%), NPA = 96.2% (CI = 95.5%-96.9%), TPA = 96.3% (CI = 95.6%-97.0%); kappa = 0.75]. Agreement after three-tiered ELISA algorithm-based discrepant result resolution: MTTT-A [PPA = 97.6% (CI = 95.6%-99.7%), NPA = 99.1 % (CI = 98.7%-99.4%), TPA = 99.0% (CI = 98.6%-99.3%); kappa = 0.93]; MTTT-B [PPA = 99.2% (CI = 98.0%-100.3%), NPA = 98.2% (CI = 97.7%-98.7%), TPA = 98.3% (CI = 97.8%-98.7%); kappa = 0.89].

Conclusion: To our knowledge, this study represents the first multicenter prospective STTT/MTTT evaluation for several ELISAs currently FDA-cleared as first tier screening tests. These data are consistent with previously-published studies utilizing banked sample cohorts, and support the conclusion that MTTT results are in statistical

agreement with the STTT algorithm. In addition to the three-tiered ELISA testing, retrospective review of patient charts is ongoing, with the goal of providing a more clinically-based resolution of discrepant STTT/MTTT results.

A-121

Commutability Study between Abbott Alinity and ARCHITECT Family of Analyzers: A Suite Solution

W. R. Stevens[1], R. S. Wonderling[2], J. J. Hupp[1]. [1]*Saint Francis Health System, Tulsa, OK,* [2]*Abbott Laboratories, Abbott Park, IL*

Background: Commutability of results across multiple platforms is one of the most important factors to consider when evaluating automated solutions for large Integrated Delivery Networks (IDNs), Health Systems, or hospitals where scalable analyzers are necessary for patient care and universal reference ranges are desired. The goal of this study was to evaluate the commutability of quality control (QC), linearity, and patient sample results between the Abbott Alinity and ARCHITECT family of analyzers in the Core Lab compared to other ARCHITECT systems placed in hospitals and clinics around the network. **Methods:** The results generated from five Alinity c instruments and one Alinity i instruments were compared to the results generated from ARCHITECT instruments. A total of 32 clinical chemistry assays and 13 immunoassays were compared using 25 to 40 samples for each set of comparison of platforms for each assay. Data was analyzed to determine the correlation coefficient and average bias. Slope and intercept was also determined using Deming regression. **Results:** The data comparison for the 32 clinical chemistry assays indicated that 90% of the correlation coefficients were 0.9924 or greater and 90% of the slopes from Deming Regression were between 0.926 and 1.043. The average bias for 90% of the chemistry assays was between -5.534% and 4.801%. The data comparison for the 13 immunoassays indicated that 90% of the correlation coefficients were 0.9932 or greater and 90% of the slopes from Deming Regression were between 0.969 and 1.13. The average bias for 90% of the immunoassays was between -3.29 and 4.1 The data demonstrated excellent commutability of results between the entire suite of Abbott Alinity and ARCHITECT analyzers for clinical chemistry and immunoassay analytes studied. **Conclusion:** Commutability of results should be carefully evaluated when comparing analyte method performance across multiple platforms and in the reference range decision process. As large delivery networks, including health systems, hospitals, and clinics move toward integrated medical records and the coverage of large populations, commutability of results is critical as patients can present at a multitude of locations. The management of reference ranges and trending of patient data is made much simpler when a single reference range can be used across the system.

A-122

A New and Improved Chemiluminescent Substrate

G. Melkamu[1], P. Kilmartin[1], J. Koti[1], M. Sandison[2], R. De Silva[3]. [1]*Beckman Coulter, Inc., Chaska, MN,* [2]*Lumigen Inc, Southfield, MI,* [3]*Lumigen Inc, Southfiled, MI*

Background: Beckman Coulter is developing a new immunoassay system that will run current Access immunoassays as well as additional new menu. Goals for this new system include improved turn-around-times for all assays, thereby meeting STAT test requirements while improving overall platform throughput. A key component of the new system is a new chemiluminescent substrate employed to generate the light signal response. This new substrate is composed of a buffered surfactant enhancer system supporting an ALP-sensitive acridan. When the acridan is triggered in-situ, it forms a dioxetanone which immediately decomposes and emits light.

Methods: Luminometer read time was assessed by determining the change in relative light unit (RLU) signal over 32 to 72 seconds using an ALP-based enzyme test method and several commercialized Access immunoassays. Improved signal-to-noise performance was demonstrated by comparing calibration curves from several sensitive immunoassays generated using Lumi-Phos 530 with curves generated with the new chemiluminescent substrate. The impact of interference from endogenous ALP was determined by assessing a panel of patient samples previously identified to contain these interferents, using assays tested with both substrates.

Results: Luminometer read time is approximately 5 minutes shorter for the new substrate than for Lumi-Phos 530. Three- to six-fold increases in signal-to-noise performance were demonstrated across the assays. Samples with known endogenous ALP interferents displayed greater than 50% reduction in spurious elevations when using the new substrate as compared to the values observed with the same samples using Lumi-Phos 530.

Conclusions: The new substrate has been optimized to generate signal rapidly, improve signal-to-noise performance, and reduce interference from endogenous alkaline phosphatase (ALP) in comparison to Lumi-Phos 530. This new substrate presents the opportunity to significantly shorten the time to first result while simultaneously improving assay sensitivity.

A-123

International Consensus on Antinuclear Antibody Patterns (ICAP) - Using Proposed Codes and Nomenclature in a Large Sample of ANA Tests Pre-Evaluated according to Brazilian Consensus on Autoantibodies (BCA)

W. S. Santos[1], D. C. Félix[1], J. B. Meireles[1], I. C. S. Melo[2]. [1]*Sabin Diagnostic Medicine, Brasília-DF, Brazil,* [2]*Unievangélica University, Anápolis-GO, Brazil*

Background: The ICAP recognizes three major groups of staining patterns in the antinuclear antibody (ANA) indirect immunofluorescence (IIF) test on HEP-2 cells: nuclear, cytoplasmic, and mitotic. All patterns were codified using an AC (*anti-cell*) code that ranges from AC-0 to AC-29. **Methods:** ANA results of serum samples referred to the laboratory, between January and June of 2017, for autoantibodies detection by IIF on HEp-2 cells were reassessed with the aim of applying the nomenclature and codes proposed by the ICAP, describing the most frequent staining patterns and comparing to the BCA. Routine ANA testing were carried out according to manufacturer's instruction. **Results:** 54,990 serum samples were tested for ANA in the period mentioned. 20.9% (11,478) were positive at a titer≥1/80 and 79.1% (43,512) were negative. Among the positive samples, 91,1% (10,473) displayed a single ICAP code and 8,8% (1,005) a composite one (multiple ICAP codes). Of these 10,473 sera with a single pattern, 85.7% presented nuclear pattern (AC1-AC14, AC-29); 4% cytoplasmic (AC-15-AC-23) and 1,5% mitotic (AC-24-AC-27). The patterns most observed in the nuclear group were: Fine speckled (AC-4) 44%; Dense fine speckled (AC-2) 24.9%; Homogeneous (AC-1) 8% ; Large/coarse speckled (AC-5) 3.9%; Nucleolar (AC-8,9,10) 2,9% and Centromere (AC-3) 2.3%. A nuclear pattern recognized only by the Brazilian Consensus (BCA), named *Quasi*-Homogeneous (*Q*H) was reported in 11.7% of the samples. The other nuclear patterns were reported at frequency lower than 1%. The cytoplasmic patterns identified were: Mitochodrion-like (AMA) (AC-21) 44.2%; Cytoplasmic dense fine speckled (AC-19) 11.6%; Discrete dots (AC-18) 10.8%; Fibrilar (AC-15,16,17) 9.2%; Fine speckled (AC-20) 8.8%; Golgi (AC-22) 8.6% and Rods and rings (AC-23) 6.7%. In the mitotic group: Nuclear mitotic apparatus NuMA (AC-26); Spindle fibers (AC-25); Centrossome (AC-24) and Intercellular bridge were seen in 56.3; 28.4; 9.1 and 6.2% respectively. The composite patterns (two or more ICAP code in a same test) were observed in 1,005 (8.8%) samples. Mixed patterns recognized only by the Brazilian Consensus were adjusted to the ICAP proposal using the correspondent codes combination. **Conclusion:** AC-4 and AC-2 were the most frequent patterns observed in this large sample studied. The nuclear *Q*H, only recognized by the Brazilian Consensus, is the third most frequent pattern observed. AMA and NuMA are the most observed patterns in the cytoplasmic and mitotic group, respectively.

A-124

Evaluation of Seven Clinical Chemistry Assays in Lipid Panel on ARCHITECT® *c* Instrument

S. Ning, H. Guo, C. Lee, P. Yin. *Abbott Diagnostics Division, China R&D, Shanghai, China*

Background: Lipoproteins and cholesterols are important biomarkers for cardiovascular disease. their levels in serum/plasma can predict risk of for coronary heart disease, which is the leading cause of death in the world. To address increasingly specific needs in for Chinese patients, seven clinical chemistry assays of lipid panel have been optimized for evaluation on ARCHITECT® *c* instrument.

Methods: The following seven analytes were evaluated: small dense low-density lipoprotein cholesterol (sd LDL-C), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (CHO), triglyceride (TG), apoprotein A1 (Apo A1), and apoprotein B (Apo B). Reagent kits from Beijing Strong Biotechnologies Inc. (BSBE) were evaluated on the ABBOTT ARCHITECT® *c* instrument. Precision, LoQ, linearity range, interference, and open on-board stability were evaluated with guidance from CLSI documents including EP15-A2, EP6-A, EP17-A2, EP7-A2, and EP 25-A. Correlation to other commercial kits was performed by using fifty human serum samples across the measuring range of each analyte.

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

Analytes	Precision (total CV)	Linearity (coefficient factor: r)	LoQ	Method Comparison (correlation factor: r)
sd-LDL-C	1.1%	0.9999	0.23 mg/dL	0.9992
HDL-C	0.6%	0.9994	0.004 mmol/L	0.9843
LDL-C	1.0%	0.9997	0.005 mmol/L	0.9992
CHO	0.5%	0.9998	0.020 mmol/L	0.9999
TG	0.7%	0.9997	0.001 mmol/L	0.9997
APO A1	1.3%	0.9996	0.001 g/L	0.9960
APO B	0.7%	0.9999	0.006 g/L	0.9960

Conclusions: These initial results of optimized assays exhibit good precision, stability, linearity, interference, and sensitivity performances on ARCHITECT® *c* instrument. In addition, they also showed excellent correlation with commercial assay kits on market. It represents these seven clinical chemistry assays of lipid panel are acceptable and applicable on ARCHITECT® *c* instrument.

A-125

Mixed Staining Patterns in a Large Sample of Sera Referred for Antinuclear Antibodies (ANA) Testing on HEp-2 cells

W. S. Santos[1], D. C. Félix[1], J. B. Meireles[1], I. C. S. Melo[2]. *[1]Sabin Diagnostic Medicine, Brasília-DF, Brazil, [2]Unievangélica University, Anápolis-GO, Brazil*

Background: The international consensus on ANA patterns (ICAP) defined 30 codes and the nomenclature for the staining patterns observed in ANA indirect immunofluorescence test on HEp-2 cells. Three major groups of staining patterns are recognized: nuclear, cytoplasmic and mitotic and the assigned codes range from anti-cell (AC) 0 to 29. Mixed pattern is the term used for the several combinations of codes that could be observed in the routine ANA testing. The aim of this study was to describe the most prevalent combinations of ANA patterns on HEp-2 cells. **Methods:** Routine ANA testing results reported in the period of January and June of 2017 were reclassified using ICAP recommendations. ANA tests were carried out according to the producer's instructions. Visual pattern recognition was done by experts on ANA readings. **Results:** In the six months period studied, 54,990 samples were tested for ANA and 11,478 (20.9%) were positives at titer≥1/80. Of these positive sera, 1,005 displayed a mixed HEP-2-IFI pattern, combining 2, 3 or 4 AC code. Double, triple and quadruple mixed pattern were observed in 98%, 1.8% and 0.2% respectively. The most common double patterns described were: AC-4(Fine speckled)+AC-6,7(Discrete dots) (n=262); AC-2(Dense fine speckled)+AC-6,7 (n=198); AC-4+AC-8,9,10(Nucleolar) (n=129); AC-3(Centromere)+AC-4 (N=125). The nuclear staining group was present in 98.5% of the combinations. Mixed patterns without nuclear staining were restricted to 15 samples. **Conclusion:** Less than 10% of positive results in routine ANA testing displayed a combination of ANA patterns. The double nuclear pattern were by far the most observed mixed pattern. Among these, AC-4+AC-6,7; AC-2+AC-6,7; AC-4+AC-8,9,10 and AC-3+AC-4 were the most specific combinations reported.

Tuesday, August 6, 2019

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

Clinical Translational Science

A-126

Upregulation of sCD14 Levels in Severe Aseptic Inflammation Induced by Continuous, Prolonged, Brisk Exercise May Reflect Athletes' Possible Endotoxemia

I. Papassotiriou[1], K. Skenderi[2], M. Papastamataki[1], D. Afordakou[1], C. Konstantellou[1], A. Haliassos[3], M. Tsironi[4]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Laboratory of Nutrition and Clinical Dietetics, "Harokopio" University, Athens, Greece, [3]ESEAP, Athens, Greece, [4]Department of Medicine, University of Peloponnesus, School of Nursing, Sparta, Greece*

Background: The "Spartathlon" ultradistance foot race, (246Km continuous, prolonged, brisk exercise for up to 36h), which physical strain renders it an ideal study model of long-term severe physical stress. The runners endure dramatic systemic and inflammatory changes, as their immune system functions intensively to cope with heart and skeletal muscle and other organ damage secondary to excessive physical strain. We have previously reported that this prolonged strenuous exercise induces systemic inflammation and endothelial activation reaching conditions seen in major trauma, septic shock, or a near-death state. Cluster of differentiation 14 (CD14) is a glycoprotein expressed on the surface of monocytes and macrophages, although low levels are also found on neutrophils and acts as a myeloid differentiation marker. CD14 can exist as a GPI-anchored membrane protein or as one of two soluble isoforms that can be generated either by cleavage from the surface of the cell or released from intracellular pools. CD14 is a co-receptor for the lipopolysaccharide (LPS)/LPS binding protein (LBP) complex, which activates a series of signal transduction pathways and inflammatory responses and leads to SIRS. Moreover, CD14 does not bind only LPS; it can also bind Gram-positive cell wall components, LAM and HSP60, as well as endogenous lipids. Upon cell activation, CD14 surface expression decreases on monocytes and sCD14 is released. Although release of sCD14 is often accompanied by a decrease in surface expression of CD14, this is not always the case. This suggests that sCD14 can be generated by a mechanism other than cleavage from the cell surface. **Methods:** In this context we aimed to investigate sCD14 expression in 24 "Spartathlon" athletes before (Phase I), at the end (Phase II) and at 48 h post-race (Phase III). Along with common blood chemistry parameters, we measured levels of IL-6, serum amyloid A protein (SAA), C-reactive protein (CRP), and Procalcitonin (PCT) and sCD14. sCD14 levels were measured by means of a chemiluminescent enzyme immunoassay (CLEIA) using an automated immunoassay analyzer, PATHFAST, (Mitsubishi Chemical Europe GmbH). **Results:** We found that: CRP, SAA and IL-6 were dramatically increased at Phase II (116-, 150- and 10,470-fold increase of the mean values, respectively), with CRP and SAA levels remained high also in Phase III (p<0.001), while PCT levels remained practically unchanged within normal range in all measured phases (p>0.950). sCD14 levels (mean±SEM) increased significantly from 189.2±33.4pg/mL at Phase I to 1,102.4±394.2pg/mL at Phase II (p<0.001) and decreased significantly at Phase III (307.1±62.4pg/mL), (p<0.01). **Conclusions:** During ultradistance exercise, transient immunosuppression and inflammatory alterations are observed as well as the regulation of lipid and carbohydrate metabolism, mitochondrial biogenesis, oxidative stress, and dehydration. These alterations accompanied by significant endotoxemia, in which pathogens or endotoxins are able to cross the intestinal barrier into the bloodstream, causing a disruption of the immune system-microbiota homeostasis. Upregulation of sCD14 levels after "Spartathlon" ultradistance foot race possible reflects endotoxemia, and may be serves as a biomarker of the degree of endotoxemia in extreme sports conditions.

A-127

Assessment of Bone Marrow Activity in Patients with Sickle Cell Disease by Soluble Transferrin Receptors Measurements: Correlation with Markers of Erythropoiesis, Iron Metabolism and Hydroxyurea Treatment

I. Papassotiriou[1], S. Zaliou[2], V. Tsaousis[2], K. Pantelaki[1], K. Larissi[3], E. Voskaridou[3]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Medicon Hellas, Gerakas, Greece, [3]Thalassemia and Sickle Cell Disease Center, "Laiko" General Hospital, Athens, Greece*

Background: Sickle cell disease (SCD) is an inherited hemoglobinopathy characterized by pathological polymerization of hemoglobin, increased red cell rigidity and poor microvascular blood flow with consequent tissue ischemia and infarction. Thus, hemolytic anemia, vaso-occlusion and vasculopathy are the hallmarks of its clinical presentation. The transferrin receptor (TfR) mediates the transport of iron into cells and the circulating TfR can be measured as soluble transferrin receptor (sTfR). sTfR levels are frequently used to establish the diagnosis of iron deficiency anemia, especially in the context of inflammation, but they also reflect bone marrow erythropoietic activity (BMA) and mass. Erythropoietic activity has been found to be the most important determinant of sTfR levels. In this context, we aimed to study and evaluate bone marrow activity in patients with compound heterozygous SCD and beta-thalassemia (HbS/βthal) based in sTfR measurements and explore possible correlations with of key features of the disease, such as the hemolytic component, hydroxyurea therapy, inflammation, along with other biomarkers of erythropoiesis and iron metabolism such as Placental Growth Factor (PlGF), Growth Differentiation Factor-15 (GDF-15), Ferritin and Hepcidin-25.

Patients and Methods: Ninety adult Caucasian patients with HbS/βthal [49 patients under hydroxyurea -a Ribonucleotide Reductase Inhibitor- (HU+) treatment and 41 patients without hydroxyurea (HU-) treatment], were included in this study, while 22 apparently healthy individuals of similar age and gender served as controls. None of the patients has received any transfusions at least 6-monthes before enrollment in the study. Along with hematologic and blood chemistry parameters determination, levels of circulating sTfR, PlGF, GDF-15 and Hepcidin-25 were measured in patients with HbS/βthal and controls using RUO and IVD immunoenzymatic techniques. BMA activity was calculated from the established formula: *patient-sTFR/meanControl-sTFR.*

Results: We found that sTfR levels were markedly elevated in all patients with HbS/βthal compared to controls (4.8±2.2 vs. 1.0±0.2 mg/L, p<0.001), resulting in a 1.6-11.9 fold increase of BMA. BMA correlated significantly with the markers of the erythropoietic and hemolytic component such as: Hemoglobin (r=-0.434, p<0.001); Reticulocyte Production Index (r=0.645, p<0.001); LDH (0.570, p<0.001); Billirubin (0.540, p<0.001) and PlGF (0.597, p<0.001), while no correlation was found between BMA and HbF levels. Furthermore, BMA values correlated significantly only with GDF-15 (0.466, p<0.001), while interestingly no correlation was found between BMA and Ferritin and Hepcidin-25 levels (r=0.101, p>0.351 and r=-0.043, p>0.710, respectively). Regarding hydroxyurea treatment no differences were found in BMA (p>0.434).

Conclusions: Our findings demonstrate that all patients with HbS/βthal studied have a significantly increased degree of erythroid BMA as assessed by measurements of sTfR levels. Furthermore, BMA is not related to hydroxyurea therapy and/or iron metabolism parameters in these patients. This implicates a likely complex action of hydroxyurea, which causes intermittent cytotoxic suppression of erythroid progenitors and cell stress signaling, which then affects erythropoiesis kinetics and physiology, leading to recruitment of erythroid progenitors with increased HbF levels, although the number of erythroid progenitors, the main source of sTfR, remains stable.

A-128

CRISPR/Cas-Mediated Generation of Mutant Mouse Models with Alzheimer's Disease Associated Mutations

K. K. H. T. T.C.S.. *Waseda University, Tokyo, Japan*

Background: Alzheimer's disease (AD) is the most common cause of dementia in the world. Some AD cases with inherited form of the disease carry mutations in the presenilin proteins (PSEN1 and PSEN2) or in the amyloid precursor protein. *PSEN1* is the most frequent causal gene in inherited form of AD, in which more than 250 mutations are identified. However, the functional consequences of each mutation still remain uncovered *in vivo*.

Methods: Here we introduced deletion of exon 9 (delta E9) mutation into the mouse *Psen1* gene utilizing CRISPR/Cas9 technology in order to explore the physiological

functions of exon 9 deficiency in Psen1 protein *in vivo*. We first designed two sgRNAs on intron 8 and intron 9, respectively to delete 885 bp including exon 9. We next constructed the all-in-one vector expressing Cas9 along with sgRNA, and performed in vitro screening of sgRNAs to induce efficient cleavages in Neuro2a cells. After confirming a large deletion caused by the selected sgRNAs in cells, both sgRNAs along with SaCas9 mRNA were injected into the cytoplasms of 250 mouse zygotes via microinjection.

Results: CRISPR/Cas-mediated genome editing resulted in the efficient generation of the 22 mutant mice (33.8%) which harbored exon 9 deletion of 65 neonates in F0 generation. Unexpectedly, one mouse (1.5%) showed complete deletion of exon 9 with high level of mosaicism (90.1 %) for additional exon 8 deletion. Deletion of exon 8 and exon 9 in *Psen1* mRNA of this mouse was also confirmed by RT-PCR. Although it has been reported that some of *Psen1* knock-in mice with point mutations exhibited embryonic lethality in a homozygous state, the delta E8-9 mouse was viable and displayed no developmental defects except for tail deformity.

Conclusion: These results indicate that CRISPR/Cas system allow the efficient generation of mutant animal models with disease-associated mutations including large deletion, and is highly useful to elucidate functional consequences of the mutations *in vivo*. CRISPR/Cas-mediated genome editing might efficiently discover novel genetic variants involved in the AD pathology.

A-129

Plasma Levels of Fibrinogen at Postoperative Day 1 is Associated with the Survival of Patients Undergoing Orthotopic Liver Transplantation for Hepatocellular Carcinoma

R. Guo, L. Xiao, X. Liu, L. Xie, Y. Chen. *Department of Laboratory Medicine, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: Hepatocellular carcinoma (HCC) is an aggressive malignancy and the second leading cause of cancer-related deaths worldwide. The prevalence of cirrhosis among HCC patients was approximately 80%. Orthotopic liver transplantation (OLT) has emerged as the optimal treatment option for patients with unresectable HCC and liver cirrhosis. Since the liver plays a central role in the regulation of hemostasis, patients undergoing OLT frequently have multiple alterations in their hemostatic system. However, few studies have illuminated the relationship between dynamic change of hemostatic parameters and prognosis after OLT. We aimed to clarify the prognostic value of hemostatic parameters for predicting the survival of patients undergoing OLT.

Methods: 182 consecutive adult patients underwent OLT for HCC between June 2012 and December 2015 at the First Affiliated Hospital of Zhejiang University were analyzed retrospectively using univariate and multivariate methods. All patients were transplanted in classic or modified piggyback fashion using well-described standard techniques Postoperatively, all patients were treated at a single intensive care unit (ICU) with standardized ICU treatment. Patient's preoperative characteristics and laboratory variables, perioperative management and surgical procedure, postoperative laboratory tests (Postoperative Day [POD] 1 to PODs 7) in all patients were retrieved from the hospital electronic medical records system. The laboratory variables included a-fetoprotein, creatinine, total bilirubin, international normalized ratio, prothrombin time, activated partial thromboplastin time, plasma fibrinogen levels, D-Dimer, and platelet count.

Results: Overall, 1-year and 2-year patient survivals were 65.9 % and 50.5%. Predictors of 2-year mortality included size of largest tumor, ascites, intraoperative blood loss, intraoperative transfusion of fibrinogen concentrate, and plasma levels of fibrinogen at POD 1 ($p < 0.05$ all), with ascites and plasma levels of fibrinogen at POD 1 as independent parameters. As the fibrinogen levels at POD 1 increased, the 1-year and 2-year survival rate were both gradually elevated. The fibrinogen levels at POD 1 showed significant correlation with patient's survival on ROC curves. The area under ROC curve was 0.753 ($p < 0.001$) with a sensitivity of 83.9 % and a specificity of 61.7 % under the cutoff value of 1.93 g/L for 1-year survival, while the area under ROC curve was 0.623 ($p = 0.003$) with a sensitivity of 77.8 % and a specificity of 41.3 % under the cutoff value of 2.23 g/L for 2-year survival. Kaplan-Meier survival analysis showed that the higher the fibrinogen on postoperative day 1 levels, the better the 1- and 2-year survival ($p < 0.001$).

Conclusion: Monitoring of the haemostatic system parameters in liver transplantations is of great importance. Plasma levels of fibrinogen at POD 1 is an independent predictor of 2-year post-OLT survival for HCC patients and may be helpful in intraoperative management, outcome prediction and decision-making.

A-130

A Statistical Model for Restoration of Serum Potassium Level Disturbed by Hemolysis

w. zhang, D. Price. *The Peter Doherty Institute, Melbourne, Australia*

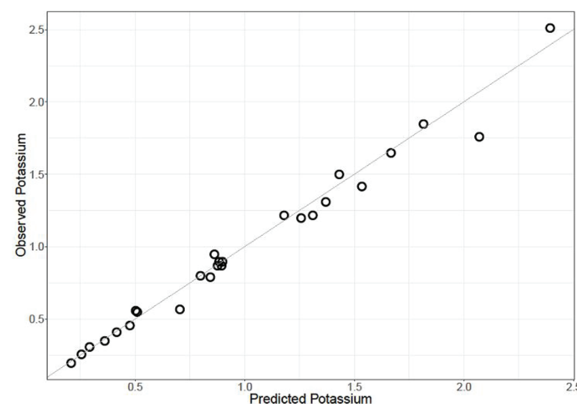
Background: Blood sample hemolysis affects pre-analytical quality and may cause pseudohyperkalemia. We established a statistical model to estimate the corrected potassium (K^+) in serum.

Methods: Serum K^+ and H index were analyzed, and blood cell index was obtained from the examined Full Blood Examination results. A linear-regression model was developed using H index, K^+ and covariates of blood cell index from 139 cell lysates of blood samples. The model was then validated against 26 *in vitro* physically hemolyzed serum samples.

Results: The final model selected H index, hemoglobin concentration (HGB), and hematocrit (HCT) as important predictors in estimating the K^+ content. The model was validated against artificially hemolyzed serum samples, which returned a correlation of 0.942 between observed and predicted net K^+ increase by hemolysis. The predictors H index, HCT, and HB contributed 93.7%, 3.5% and 2.8% to the model R^2, respectively.

Conclusion: *In vitro* hemolysis induced pseudohyperkalemia could be accurately predicted and restored by our model for clinical application.

Figure 1 Validation of the statistic model with 26 artificially hemolyzed blood samples on net increases of [K+]



A-131

Symmetric Dimethylarginine as an Alternative Marker for Estimation of Glomerular Filtration Rate

V. Nandakumar, J. Hornseth, C. Giesen, L. Donato, J. Lieske, J. Meeusen. *Mayo Clinic, Rochester, MN*

Background: Symmetric dimethylarginine (SDMA) is a small (202 Da) biologically inert byproduct of metabolism. It is produced by all cells during proteolysis of methylated proteins, eliminated by glomerular filtration, and hence proposed as a potential GFR biomarker. The objective of this study was to compare glomerular filtration rate measured by iothalamate clearance (mGFR) to GFR estimated (eGFR) using SDMA, creatinine, or Cystatin (CysC) in cohorts of patients with and without kidney disease.

Methods: SDMA, creatinine and CysC were measured in biobanked residual plasma from 99 subjects with available mGFR results. Clinical indications for mGFR were: potential kidney donation (n=20), post kidney donation (n=20), kidney transplant recipient (n=20), liver transplant recipient (n=20) and chronic kidney disease staging (n=19). Iothalamate renal clearance was used to assess mGFR. SDMA was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Creatinine was measured using the Roche enzymatic method on a Cobas c701. CysC was measured on a Cobas c501 by immunoturbidimetric method (Gentian Diagnostics, Moss, Norway). A Multivariate linear regression was used to develop predictive models for mGFR. Multivariate models included log-transformed- SDMA, or creatinine or CysC combined with age and sex as additional covariates.

Results: A model using SDMA and clinical demographics accurately predicted mGFR, and performed similarly to models that employed CysC or creatinine (Table). For all biomarkers, log transformation and including age, sex and disease cohort yielded increasingly improved fit. When examined by disease group, SDMA and

CysC overall performed best with SDMA better in kidney transplant patients and CysC better in post kidney donation subjects.

Conclusions: In this pilot study SDMA was a promising biomarker of GFR across disease groups, performing comparably to CysC and better than creatinine. SDMA may have particular advantages in post kidney transplant patients, a population where close monitoring of GFR is essential.

Table: Predictive models for mGFR using X= SDMA, Creatinine, CysC

	R^2 FOR MODEL FIT		
Model components	**SDMA**	**Creatinine**	**CysC**
X, Age, Sex	0.62	0.49	0.53
Log_{10}(X), Age, Sex	0.64	0.65	0.73
Log_{10}(X), Age, Sex, Disease group	0.74	0.72	0.77
Log_{10}(X), Chronic kidney disease only	0.75	0.51	0.72
Log_{10}(X), Post kidney donation only	0.11	0.28	0.48
Log_{10}(X), Liver transplant recipient only	0.45	0.41	0.65
Log_{10}(X), Kidney transplant recipient only	0.75	0.59	0.59
Log_{10}(X), Potential kidney donation only	0.34	0.18	0.36

A-132

Hepatic Proteomic Alterations in Signaling Pathways Associated with Inflammation Development and Resolution in Patients with Severe Alcoholic Hepatitis

J. Hardesty[1], D. Warner[1], J. Jacobs[2], C. McClain[1], I. Kirpich[1]. [1]*University of Louisville, Louisville, KY,* [2]*Pacific Northwest National Laboratory, Richland, WA*

Background/Objectives: Alcoholic liver disease (ALD) encompasses a broad spectrum of alcohol-induced liver disorders ranging from fatty liver to more severe forms including alcoholic hepatitis (AH), cirrhosis, and hepatocellular carcinoma. ALD is a growing concern due to an insufficient understanding of the disease pathogenesis, limited treatment options, and high short-term mortality in patients with severe AH (sAH). Infection occurs in ~25% of sAH cases, and is a major factor contributing to death. Systemic inflammatory responses caused by infection are a hallmark of sAH. In the present study, we performed a proteomic analysis of liver tissue obtained from patients with sAH with the goal of identifying changes linked to critical signaling pathways that regulate the inflammatory responses associated with sAH as well as the subsequent resolution of inflammation network. **Methods:** Liquid chromatography and tandem mass spectrometry analyses were performed to measure proteomic changes in explanted liver tissues obtained from sAH patients (n=6, 44.1±4.4 *y.o*., 4 M/2 F, mean Model for End-Stage Liver Disease score (MELD)=35.7±1.78) and individuals without ALD (controls, n=12, 55.5±4.4 *y.o*., 4 M/8 F). MetaCore ontology analyses (false-discovery rate < 0.05) were performed to identify changes in metabolic/signaling pathways altered in sAH patients. **Results:** Of 7296 identified proteins, 788 were differentially expressed between sAH and control liver tissue (405 up- and 383 down-regulated, respectively, cut off 1.5-fold change, p < 0.05). MetaCore analyses identified several processes significantly altered in livers of sAH patients, including inflammation. The proteins of these networks are CCL21, EpCAM (chemotaxis and infiltration, *p*=1.8e-7), NFκB1, NFκB2, STAT6 (inflammatory transcription factors, *p*=0.0001), IL-18, IL-16, IL-32 (cytokine release, *p*=2e-4), CTSS, CTSV, and S100P (neutrophil degranulation, *p*=2e-4). Proteins involved in phagocytic clearance of bacteria were underrepresented in sAH patients. These included MARCO, CD209, CD302 (PAMP receptors, *p*=4.2e-8), and SOD1 (oxidative burst, *p*=4.9e-4). Inflammatory resolution pathways such as dead cell clearance, anti-inflammatory factors, and tissue remodeling were also underrepresented in sAH. Proteins diminished in inflammatory resolution included STAB2, CD169, MST1 (dead cell clearance, *p*=4.9e-4), IL1RN (IL1β signaling inhibition, *p*=0.001), and SERPINA3 (protease inhibition, *p*=0.003). Importantly, ANXA1, a key regulator of both of the innate and adaptive immune system, and a well-known pro-resolution factor was significantly up-regulated in sAH (p=3.8e-06), however FPR1, its receptor, was decreased in AH (*p*=0.002), suggesting compromised resolution of the inflammatory responses *via* this pathway. Lastly, metabolic pathways of biotransformation/detoxification and cell defense against reactive oxygen species were altered in sAH livers. An important xenobiotic/drug-metabolizing enzyme, CYP3A4, was significantly downregulated in sAH patients (p=0.017). In addition, expression of numerous members of the glutathione transferase and sulfotransferase families were decreased in sAH compared to controls, including GST1 (p=0.002), GST2 (p=0.004), GSTM1 (p=0.02), and SULT1A1 (P=0.0004), and SULT2A1 (p=0.001), respectively.

Conclusions: Liver proteomic analysis identified alterations in multiple signaling pathways that may contribute to the development of progressive liver injury in sAH patients, including activated pro-inflammatory and defective anti-resolution pathways, and diminished antimicrobial defense and xenobiotic detoxification. Further studies are required to identify downstream mechanisms, potential novel biomarkers, and therapeutic targets.

A-133

Effects of Delta-Tocotrienol Supplementation on Biochemical Markers in Patients with Non-Alcoholic Fatty Liver Disease

D. A. khan, M. A. Pervez, A. Ijaz, A. U. Slehria. *NUMS, Rawalpindi, Pakistan*

Background: Non-alcoholic fatty liver disease (NAFLD) is a growing public health problem worldwide and will probably emerge as the leading cause of end-stage liver disease. Inflammation, increased oxidative stress and insulin resistance (IR) are the main factors for development of NAFLD. Delta-tocotrienol (δ-tocotrienol) has potent anti-inflammatory, antioxidant and anti-insulin resistance properties and may reduce liver injury in NAFLD. The aim of this study was to examine the effects of δ-tocotrienol supplementation on biochemical markers in patients with NAFLD.

Methods: In this randomized, placebo-controlled trial, 71 patients aged >20 years, having ultrasound-proven fatty liver, with a fatty liver index (FLI) of ≥ 60 and persistent elevation of alanine transaminase (ALT) were included. The patients were randomized to receive lifestyle recommendations (low-fat diet in conjunction with increased physical activity) plus either 600 mg δ-tocotrienol (n=35) or placebo (n=36) for 24 weeks. Blood lipid profile, liver function tests, anthropometric, inflammatory and oxidative stress indices, as well as insulin resistance and hepatic steatosis scores were measured at the beginning and the end of the study, and compared between and within groups.

Results: Out of 71 enrolled patients, 62 patients (mean age 44.2 ± 8.7; 27 males and 35 females); 30 in the δ-tocotrienol group and 32 in the placebo group, completed the study. C-reactive protein, interleukin-6 (IL-6), tumor necrosis factor (TNF-α), malondialdehyde (MDA) and homeostasis model of insulin resistance (HOMA-IR) decreased significantly only in tocotrienol group. Triglycerides (TG), γ-glutamyltransferase (γ-GT), ALT and FLI decreased significantly in both groups; however, there was a significant difference between two groups (p< 0.05).

Table 1 Comparison of biochemical markers between the groups at week 24[a]

	δ-tocotrienol (n=30)		Placebo (n=32)		
Analyte	**Baseline**	**24 week**	**Baseline**	**24 week**	***P***[b]
TG (mmol/L)	2.44 ± 0.5	2.07 ± 0.47	2.45 ± 0.46	2.27 ± 0.45	0.001
γ-GT (IU/L)	51.13 ± 8.93	41.40 ± 9.60	50.09 ± 9.3	45.12 ± 9.80	<0.001
ALT (IU/L)	82.96 ± 18.1	65.86 ± 17.7	83.83 ± 17.8	77.12 ± 16.5	<0.001
hs-CRP (mg/L)	4.28 ± 1.53	3.25 ± 1.26	4.44 ± 1.50	4.32 ± 1.37	<0.001
IL-6 (pg /mL)	14.32 ± 3.80	10.20 ± 3.72	14.10 ± 3.74	13.88 ± 3.30	<0.001
TNF-α (pg /mL)	15.61 ± 5.46	11.87 ± 3.26	14.62 ± 5.53	14.24 ± 4.49	0.001
MDA (µmol/L)	6.42 ± 1.45	5.00 ± 1.20	6.39 ± 1.38	6.25 ± 1.22	<0.001
Glucose (mmol/L)	5.75 ± 0.48	5.30 ± 0.40	5.7 ± 0.48	5.6 ± 0.49	0.001
Insulin(mIU/L)	13.34 ± 3.6	11.76 ± 3.09	12.99 ± 3.55	12.73 ± 3.04	0.001
HOMA-IR	3.40 ± 0.87	2.78 ± 0.74	3.3 ± 0.95	3.24 ± 0.83	<0.001
FLI	83.75 ± 9.4	70.17 ± 12.5	85.16 ± 8.6	80.80 ± 9.3	<0.001

[a]Data are presented as mean ± SD
[b]Between group comparison using ANCOVA

Conclusions: Our result showed that daily intake of 600 mg δ-tocotrienol is superior to placebo in amelioration of biochemical markers in patients with NAFLD.

A-134

Comparison of Human Whole Blood Dolutegravir Concentrations Collected on Dried Blood Spot Cards versus Mitra® Microsampling Devices

K. H. Blake[1], A. Schauer[1], C. Sykes[2], H. Bryan[1], A. D. M. Kashuba[1]. *[1]University of North Carolina at Chaple Hill, Chapel Hill, NC, [2]University of North Carolina at Chapel Hill, Chapel Hill, NC*

Background: Human whole blood collected by dried blood spots (DBS) or volumetric absorptive microsamplers (VAMS) allow for the quantitation of drug adherence through a minimally invasive procedure without the need for specialized equipment or phlebotomy. DBS is a more traditional sampling method known for its ease of collection but samples may be affected by non-homogeneity or hematocrit bias. VAMS allows for the collection of a controlled amount of blood (10uL or 20uL), eliminates the effect of hematocrit, and punching is not required. Here, we present methods that were developed to quantify the antiretroviral dolutegravir (DTG) in human whole blood collected by DBS and VAMS. Whole blood results are compared for reproducibility and bias with results generated from plasma.

Methods: For DBS and VAMS assays, calibration standards were prepared in K_2EDTA whole blood at eight concentrations to give a calibration range of 10.0-10,000ng/mL with quality control (QC) samples prepared at 30.0, 800, 8000ng/mL. For the plasma assay, the calibration range was 1-10,000 ng/mL over 12 calibrators with QC samples prepared at 3.00, 15.0, 150, 800, 8000ng/mL. Calibration standards and quality control samples were wicked onto a VAMS (Mitra® 20uL tip), spotted (50uL) onto a DBS card (Whatman 903), or prepared in plasma. Three samples (DBS, VAMS, plasma) from healthy volunteers dosed with dolutegravir were collected 5 times over 28 days. Whole blood was collected in K_2EDTA tubes and subsequently spotted onto a DBS card (50uL) and wicked onto a Mitra tip (20uL). The remainder was centrifuged to obtain plasma. Samples were extracted by protein precipitation with the internal standard dolutegravir-^{13}C, d_5. Chromatographic separation was achieved on an XTerra MS C18 (50 x 2.1mm, 3.5um) column using electrospray ionization on an AB Sciex API-5000 triple quadrupole mass spectrometer. Based on sample recovery, a correction factor of 0.55 was applied to the VAMS data. Data are presented as mean (SE).

Results: All assays met 15% (20% LLOQ) acceptance criteria for precision and accuracy. Of the samples collected, 93 sampling points had paired plasma, DBS, and VAMS result within the calibration range. All combinations were strongly correlated with p-values of <0.05 and r-values of 0.94, 0.95, and 0.98 for the comparison of VAMS vs. DBS, VAMS vs. plasma, and DBS vs. plasma, respectively. Compared to DBS, VAMS DTG concentrations were 101 (2.7)%. Compared to plasma, DBS DTG concentrations were 60% (0.73)%. Compared to plasma, VAMS DTG concentrations were 62 (1.2)%. The deviations seen between plasma relative to the DBS and VAMS are expected due to DTG partitioning primarily in the plasma.

Conclusions: Assays have been developed to analyze human whole blood collected on DBS and VAMS for DTG concentrations, and these were compared to plasma concentrations. These assays met acceptance criteria for accuracy and precision. DBS and VAMS concentrations were nearly identical, and DBS and VAMS concentrations relative to plasma were as expected indicating both VAMS and DBS are viable alternatives to plasma for assessing DTG adherence.

A-135

Oxalate Measurement in Plasma and Dialysate Using a Semi-Automated Oxalate Oxidase Method

C. D. Giesen, J. Lieske. *Mayo Clinic, Rochester, MN*

Background: Oxalate is an insoluble dicarboxylic acid, which is an end product of liver metabolism of glyoxalate and glycerate. Humans lack an enzyme to degrade oxalate, and thus it must be eliminated by the kidney. Oxalate is a strong anion and tends to precipitate with calcium, especially in the urinary tract. Consequently, about 75% of all kidney stones contain calcium oxalate in some proportion. When the glomerular filtration rate is decreased, oxalate begins to be retained in the body and in advanced chronic kidney disease can precipitate in tissues, a dire condition called oxalosis. In such patients, plasma oxalate concentrations and oxalate removal by dialysis must be carefully monitored by serial measurements of oxalate in the dialysate fluid. The current studies verified the performance of a semi-automated oxalate oxidase method for determination of oxalate in human plasma and dialysate fluid.

Methods: Oxalate was measured in acidified plasma filtrate (pH 2.3-2.7 with 12N HCl) and dialysate fluid (pH 2.5-3.0 with 6N HCl) by an enzymatic assay that employs oxalate oxidase. The reaction releases hydrogen peroxide, which in the presence of peroxidase reacts with indamine dye to give a colored end point that was measured using a plate spectrophotometer at 590 nm. To improve precision all pipetting steps were carried out using an automated liquid handler. Specifically, 15 µL of 5 mM sodium nitrite (in 0.066 M citrate buffer, pH 3.5) was added to 250 µL of standard, quality control (QC) and acidified plasma filtrate/dialysate and incubated for 5 minutes at room temperature. Nitrited standard/QC/sample (100 µL) was added to 500 µL of Trinity Biotech Oxalate Reagent A in a 48 well microplate (Corning 351178) and an initial blank-adjusted OD measured at 590 nm. Next, 40 µL of Trinity Reagent B was added and the plate incubated for 10 minutes. Blank-adjusted OD at 590 nm was again measured and results determined using a concurrently run calibration curve (oxalate 2.5-250 µmol/L).

Results: Linearity was verified by n=3 mixing studies as 1-300 µmol/L in plasma and 1-100 µmol/L in dialysate ($y=1.017x+0.067$; $R^2=0.999$ and $y=0.999x+0.209$; $R^2=0.999$, respectively). Imprecision (inter-assay) was acceptable across clinically relevant ranges in both matrices (plasma: 5.6% at 11.3 µmol/L, 2.2% at 25.4 µmol/L; dialysate: 4.4% at 9.9 µmol/L, 1.8% at 41.7 µmol/L). Oxalate recovery was acceptable in both matrices across their respective measuring ranges (plasma mean=102.8% (range=92.8%-108.9%); dialysate mean=102.2% (range=90.4%-106.5%)). Results were comparable to our laboratory's current ion chromatography method but biased slightly lower: (plasma (n=100): $y=0.8383x+1.2792$, $R^2=0.8702$, dialysate (n=6): $y=0.8875x+1.5832$, $R^2=0.997$).

Conclusion: We demonstrated that oxalate can be measured accurately and precisely across the clinically relevant ranges in human plasma and dialysate matrices using a semi-automated enzymatic assay. This relatively simple assay could potentially be employed in most central chemistry laboratories where need for this specialized testing exists.

A-136

Clinical Laboratory Data Analytics for Identification and Progression of Non-Alcoholic Fatty Liver Disease in New Mexico

T. Borunda[1], K. Swanson[1], A. Mwithi[2], M. Koenig[2], R. Feddersen[1], P. Deming[3], R. Gullapalli[4]. *[1]TriCore Reference Laboratories, Albuquerque, NM, [2]Rhodes Group, Albuquerque, NM, [3]University of New Mexico College of Pharmacy, Albuquerque, NM, [4]University of New Mexico School of Medicine, Albuquerque, NM*

Background: Non-alcoholic fatty liver disease (NAFLD) is an emerging and fast-growing cause of liver disease in the United States (US). The prevalence of NAFLD is estimated to be between 20%-30% of the general population in western countries. New Mexico has the highest liver disease associated mortality rate in the US and is the 8th overall cause of mortality in the state. There are no established drug therapies to treat NAFLD at the present time. Early identification could encourage life style modification to slow or stop the progression of NAFLD to cirrhosis. Retrospective laboratory data may be an invaluable resource to identify and screen NAFLD patients among the general population. **Objective:** Evaluate the use of temporal clinical laboratory data for the early identification and progression of NAFLD disease in patients of New Mexico. **Methods:** Retrospectively, subjects with a history of a liver biopsy from August 2013 to October 2018 with a confirmed diagnosis of NAFLD were identified from the clinical laboratory database. Additional data collected included the patient's fibrosis score and liver steatosis status. Subjects were then matched to the Laboratory Information System's (LIS) longitudinal database to extract the patient's temporal clinical laboratory results for the same five year time period. Laboratory values extracted include albumin, bilirubin, AST/ALT, HbA1c and cholesterol. The variation of albumin and cholesterol values were calculated over the duration of the study data. **Results:** Liver biopsies from 146 unique patients were identified in the laboratory database. From these liver biopsies 37% had a steatosis score of 1, 20% had a score of 2 and 15% had a steatosis score of 3. When looking at fibrosis scores, 61% of patients had a score of F0, 14% had F1, 7% had F2, 6% had F3, and 9% had F4. Our initial exploratory data analysis revealed 95% of patients with an steatosis score of 3 had normal albumin levels, ~50% had a normal HbA1C, 29% had a total cholesterol of <150 mg/dL and 18% had an ALT of <40 U/L. Among the patients who had a decrease of 5% in albumin over the duration of five years, 76% of patients developed early to severe steatosis and 34% had developed early fibrosis. All patients with a 10% decrease in albumin had severe steatosis and 40% had fibrosis at the end of the study period. **Conclusion:** The utility of clinical laboratory data analytics as determinants of population health with NAFLD is an unexplored area. Temporal clinical laboratory data has the potential to identify NAFLD patients whose liver disease is progressing in the absence of clinical evidence. Clinical laboratory databases may

serve as valuable screening tools for the implementation of population health initiatives in liver diseases.

A-137

Alterations in Plasma Lipid Metabolites in Patients with Mild and Severe Form of Alcoholic Liver Disease: Correlations with the Disease Severity

D. Warner, V. Vatsalya, C. McClain, I. Kirpich. *University of Louisville, Louisville, KY*

Background/Aims: Alcoholic liver disease (ALD) is one of the most common liver diseases in the Western world, with a pathophysiological spectrum, including steatosis, alcoholic hepatitis (AH), cirrhosis, and potentially hepatocellular carcinoma. The pathogenesis of ALD is not well understood, and there are no effective medical therapies to prevent or to treat any stage of ALD. Therefore, there is a great need to identify novel mechanisms, therapeutic targets, and reliable non-invasive biomarkers. A novel concept has been recently proposed that oxylipins, oxidative metabolites of polyunsaturated fatty acids (PUFAs), including epoxy- and dihydroxy-fatty acids (Ep-FAs and dihydroxy-FAs, respectively) play important roles in ALD pathogenesis. **The goal** of the present study was to examine alterations in n6 PUFA oxylipins in patients with mild and severe alcohol-related liver injury, and to evaluate their correlation with disease severity. **Methods and Patient Population:** Plasma oxylipins were measured by HPLC/MS in a cohort of 98 alcohol-dependent subjects. There were 48 patients without or with mild liver injury based on ALT levels (no-ALD: ALT ≤ 40 U/L (26.47±2.28 U/L), n=15 [7 M/8 F, 40.4±2.9 y/o], and mild-ALD: ALT > 40 U/L (98.55±9.82 U/L), n=33 [27 M/6 F, 44.5±1.7 y/o], respectively) and none of these patients showed clinical manifestations of ALD. We also had 50 patients with AH (32 M/18 F, 48.09±1.4 y/o). Because ALT levels do not always correlate with disease severity, we used Model for End-Stage Liver Disease (MELD) to evaluate the severity of liver injury in AH patients. MELD score is calculated based on serum bilirubin, international normalized ratio, and serum creatinine. MELD ≤ 19 and MELD ≥ 20 are considered as moderate and severe AH, respectively. Average MELD score in AH patients was 22.46±0.72. The control group consisted of 29 healthy volunteers with no history of excess alcohol consumption or liver disease (20 M/9 F, 38.1± 2.65 y/o). **Results:** Plasma oxylipin analysis revealed significantly elevated levels of 13- but not 9-hydroxy-octadecadienoic acid, an oxidized metabolite of linoleic acid (LA) in AH patients compared to controls and alcohol-dependent subjects with or without liver injury. The levels of 9,10- and 12,13-epoxy-octadecenoic acids (EpOMEs, LA-derived epoxy-Fas), were significantly lower in all alcohol-dependent groups compared to controls, suggesting that ethanol consumption, rather than the presence of liver injury, is responsible for these changes. However, a negative correlation of 12,13-EpOME with MELD score (r=-0.347, p=0.016) also suggested that low 12,13-EpOME might be a lipid biomarker for severe AH. Among dihydroxy-FAs, the most prominent change was found for 8,9-DiHETrE, an arachidonic acid-derived metabolite, which was elevated in all alcohol-dependent subjects compared to control individuals. Notably, AH patients had significantly higher 8,9-DiHETrE compared to alcohol-dependent patients with or without ALD. **Conclusions:** Our data revealed several unique changes in plasma oxylipins in alcohol-dependent individuals with or without liver injury. These lipid mediators could be new biomarkers and novel therapeutic targets for ALD, and their potential hepatotoxic or hepatoprotective effects require further investigation.

A-138

Prevalence and Risk Factors for Chronic Kidney Disease from Four Countries in Sub-Saharan Africa: An AWI-Gen Sub-Study

J. George, J. Brandenburg, J. Fabian, S. Naicker, M. Ramsay. *University of Witwatersrand, Johannesburg, South Africa*

Background: Our aim was to determine the prevalence of chronic kidney disease (CKD) in six AWI-Gen study sites from four sub-Saharan African countries. The AWI-Gen study aims to examine the long term health consequences of rapidly changing environmental and demographic conditions in the context of African genome diversity and to inform public health interventions to mitigate the increase in non-communicable diseases.

Methods: Unrelated adult participants aged 40 to 60 years were recruited from Nanoro (Burkina Faso), Navrongo (Ghana), Agincourt, Dikgale and Soweto (South Africa), and Nairobi (Kenya). Serum creatinine was measured using an IDMS traceable Jaffe reaction (Randox) and urine albumin by an immuno-turbidometric method (Cobas) at a central laboratory. Estimated glomerular filtration rate was determined by the Modification of Diet in Renal Disease (MDRD) and by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations with and without the use of the African-American (AA) ethnicity factor. Chronic kidney disease was defined by an estimated glomerular filtration (eGFR) of <60ml/min/1.73m^2 (CKD-EPI without the use of the AA factor) and/or urine albumin creatinine ratio ≥ 3mg/mmol creatinine. Comparisons for potential CKD co-morbidities were computed between individuals without CKD and with CKD. The t test was used for continuous parametric data, Fisher's exact test for binary traits (sex, diabetes, alcohol intake, HIV status, smoking, CVD history and hypertension), with the $\chi 2$ test for categorical variables that are not binary, with p value computed by Monte Carlo simulation with p value for significance as p<10^{-4} . We age/sex standardised prevalence based on African data from the United Nations World Population prospects 2017. We used logistic regression to analyse co-morbidities associated with the presence of CKD.

Results: After excluding individuals with missing data we had 8,110 participants. The use of the AA ethnicity factor resulted in higher eGFRs using both equations; we therefore limited all analyses to the CKD-EPI equation without the AA factor. The overall prevalence of CKD was 10.9% (10.2-11.8). We noted geographical differences in prevalence with a range of 14.0% (11.9-16.4) in Agincourt, South Africa, to 6.6% (5.9-7.9) in Nanoro, Burkina Faso. CKD prevalence was higher in women 12.0% (10.8-13.2) compared to men 10.0 %(9-11.2), p<0.05. The prevalence of associated common co-morbidities [hypertension, diabetes, HIV and current smoking] was higher at both South African sites compared to West African sites. At least one of the following three associated co-morbidities [hypertension, diabetes, or HIV] was present in 65% (n=588) of all CKD cases (n=877); 40% of individuals without CKD (n=6939) had at least one of these risk factors. Hypertension was the most prevalent co-morbidity, present in 52.4% of people with CKD.

Linear regression analysis showed that older age, female gender, current smoking, and the presence of one or more of the following comorbidities: hypertension, diabetes mellitus, HIV infection and hypertriglyceridaemia were independently associated with CKD in these African communities.

Conclusions: Chronic kidney disease is an important public health problem in Africa and efforts should be directed at validating the most appropriate equations to use for early detection, and to initiate preventative measures.

Word count 500

A-139

Opportunity of Real Time, Longitudinal Clinical Laboratory Data to Enhance Diabetes Disease Surveillance

T. Borunda, K. Swanson, R. VanNess, M. Dodd, M. Crossey. *TriCore Reference Laboratories, Albuquerque, NM*

Background: Currently the Centers for Diseases and Prevention (CDC) monitor the health of the population with the Behavioral Risk Factor Surveillance System (BRFSS). The BRFSS is a state based random digit-dialed, landline telephone survey of the U.S. civilian non-institutionalized population that monitors the crude prevalence of chronic diseases including diabetes. The BRFSS estimates prevalence with a series of questions around pre-diabetes and diabetes. Data collected provides annual point prevalence data. Longitudinal clinical laboratory data has the opportunity to supplement BRFSS with detailed and timely information used in the diagnosis and treatment of diabetes. **Objective**: Compare rates of pre-diabetes and diabetes from clinical laboratory hemoglobin A1c (HbA1c) results in New Mexico to BRFSS data. Evaluate the value of longitudinal laboratory data for monitoring diabetes classification and progression of pre-diabetes and diabetes. **Methods**: Subjects were selected from a list of patients with at least two HbA1c laboratory results in two different years between January 1, 2014 and December 31, 2018. Data collected included HbA1c, age, and geographic location. The HbA1c results were then analyzed and divided into four groups defined by the American Diabetes Association (ADA) guidelines; normal 4% - 5.6%, pre-diabetes 5.7% -6.4%, controlled diabetes HbA1C<7% and uncontrolled diabetes ≥7%. The values were analyzed across a 5 year period and compared to diabetes prevalence rates reported by BRFSS. Longitudinal data was also evaluated for changes between the groups. **Results**: 803,823 HbA1c values were retrieved from the 5 year study period. The prevalence of pre-diabetes and diabetes was estimated at 2.35% and 1.87% respectively compared to the BRFSS data showing 8.9% and 11.3%. Averages were similar for each year. Pre-diabetes was noted in an average of 33% of HbA1c results (range of 30.3-34.8%). Controlled diabetes accounted for only 7.1% of the results (range 6.4 to 7.7%) and uncontrolled diabetes averaged 18.5% of the results (range 17.5% to 20.1%). Longitudinal data for 158,736 unique pre-diabetic and diabetic patients was used to identify increasing, decreasing or stable HbA1c levels. The percent of pre-diabetics showed 2.9%, 4.6%, and 92.4% had increasing, decreasing or stable HbA1c levels respectively. For controlled diabetics 8.3%, 13.6% and 78.1% had increasing, decreasing or stable levels versus uncontrolled diabetics

at 26.8%, 16.4%, and 56.8%. **Conclusion**: Real time, longitudinal clinical laboratory data did not provide the same diabetes prevalence rates compared to BRFSS data. This may have been due to a patient selection bias or calculation of the denominator for the prevalence rate. Laboratory data lacked information on race/ethnicity, education level or income provided through BRFSS. However, longitudinal laboratory data provided detailed information on diabetes progression not available through BRFSS. Uncontrolled diabetics had the highest percent of change in diabetes categories defined by ADA and nearly 3.0% of the pre-diabetic patients were progressing towards diabetes. Clinical laboratory data provided more timely results from 2018, compared to the most recent 2017 file from BRFSS. Laboratory data can be useful in supplementing state surveillance reports by providing valuable disease progression trends to help clinicians, epidemiologists, and policy makers understand a population's status more effectively.

A-140

Standardization of the Isolation of Umbilical Cord Cells under Xenofree Conditions by Explant Method and Evaluation of Cell Viability after Cryopreservation

C. P. T. B. Mendonça, R. F. P. M. Finamor e Silva, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: The human umbilical cord is a promising source of mesenchymal stem cells (HUCMSCs). Unlike bone marrow stem cells, they have a painless collection procedure and faster self-renewal properties. HUCMSCs are attractive autologous or allogenic agents for the treatment of malignant and nonmalignant solid and soft cancers, due to high self-renowal capacity, multilineage differentiation potential and immunomodulatory properties. The choice of isolation method is a critical step in obtaining cells with optimal quality and yield in companion with clinical and economical considerations. The isolation can be by the enzymatic digestion technique or explant method. The technique of enzymatic digestion provides a vairable product and often it has a high cost. The explant method has advantages over the enzymatic method, since it avoids cell damage by contact with digestion enzymes, besides being a protocol of low cost. **Objective:** To develop a protocol for isolation and cryopreservation of HUCMSCs under xenofree conditions using the explant method and to assess cell viability after cryopreservation. **Methods**: Samples were obtained from of healthy donors of Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil). The umbilical cord was collected and packaged in a sterile vial, transported to the laboratory and stored in the refrigerator at 2 to 8 °C until the beginning of processing. The cell isolation protocol was performed using the explant method with Xenofree complete médium. Microbiological and *Mycoplasma* tests were performed on the cell supernatant. After the counting in Neubauer's chamber the cells were criopreserved using Mr. Frousty for 24 hours at -80 °C and then liquid nitrogen. **Results**: A total of 17 samples were processed; cells from 12 volunteers were cryopreserved at zero passage (p0). The concentration of cryopreserved cells per volunteer varied from 6.0 x 10^4 cells/vial to 2.5 x 10^6 cells/vial, with viability greater than 90%. Of the volunteers who did not have cryopreserved cells, one was a patient *Streptococcus B* positive, it was observed contamination of plaques and the material was discarded. Other patient presented severe hypothermia during delivery and then a small part of the cord was sent, the cells number being isolated very small. All thawed aliquots presented viability greater than 80% after thawing. Behind aliquots were expansed and cryopreserved with viability greater than 90%. **Conclusion**: The protocol developed proved effective for isolation and cryopreservation of HUCMSCs with high cell viability. In addition, the process under Xenofree conditions is importante in clinical applications, in which the use of animal components should be completely avoided, and it has been shown results with high cellular viability at a clinical scale. Explant method possesses several advantages for HUCMSCs isolation in research and clinical settings such as reduced price and risk of biological contaminations. These advantages can make this method as the best choice for the processing of these cells in many research and clinical projects. Further investigations are recommended to a better understand of celular events in order to optimize and design isolation step more precisely.

A-141

Prostate-Specific Antigen (PSA) Test Use in the United States for Men Age 30-64, 2011-2017: Implications for Practice Interventions

K. P. Sharma, D. Siegel. *Centers for Disease Control and Prevention (CDC), Atlanta, GA*

Background: The U.S. Preventive Services Task Force (USPSTF) has issued several recommendations discussing use of the prostate-specific antigen (PSA) test to screen for prostate cancer. In 2012, USPSTF recommended against PSA screening for all men after its previous 2008 guidance noted insufficient evidence to screen men younger than 75. Our objective was to evaluate the rate of PSA test use in different age groups from age 30 through 64 for the years 2011 through 2017 by using large insurance claims databases of U.S. men.

Methods: Claims data from employer plans for men age 30-64 were from IBM Watson® Health's MarketScan databases for commercial claims and encounters in 2011-2017 encompassing 4.2-4.7 million men. Crude rates for PSA test use in each calendar year were the proportion of included men with ≥1 PSA test(s) per 12 months of continuous enrollment. All claims with ≥1 prostate-related conditions or non-laboratory procedures were excluded. Annual percent change (APC) in PSA test use was estimated for various line segments by joinpoint regression analysis, fitting trend data to identify the log-linear model with the fewest number of inflection points. The APC estimate was the log-linear slope of the trend and associated p-value for significance of the estimate. Additionally, overall change in test use rate from 2011 to 2017 was calculated with p-value based on chi-square test.

Results: The population remaining after excluding claims with ≥1 prostate related conditions constituted 92.6%-93.1% of men in 2011-2017. Estimated PSA test use rates in 2011-2017 were: 1.36%-1.42% (age 30-34), 3.43%-4.14% (age 35-39), 11.0%-13.4% (age 40-44), 18.1%-21.0% (age 45-49), 31.2%-33.0% (age 50-54), 34.7%-36.8% (age 55-59), and 37.6%-41.2% (age 60-64) years. APC values for 2011-2017 were: -0.5% (P = 0.11) for men age 30-34, -3.0% (P = 0.001) for men age 35-39, -3.1% (P < 0.001) for men age 40-44, -2.4% (P = 0.001) for men age 45-49, -0.2% (P = 0.66) for men age 50-54, and 0.0% (P = 0.997) for age 55-59. For men age 60-64, APC was -3.3% (P = 0.054) from 2011 to 2013, and it was 1.2 % (P = 0.045) from 2013 to 2017. For men age 30-34, estimated overall PSA test use rate decreased by 2.7% (P = 0.10) from 2011 to 2017. Estimated overall PSA test use rates decreased by 16.5%, 16.9%, 13.4%, 2.1%, 1.3% and 2.1%, respectively, for men age 35-39, 40-44, 45-49, 50-54, 55-59 and 60-64 years from 2011 to 2017 (P < 0.001).

Conclusion: There is still a substantial proportion of men receiving the PSA test although the test use has decreased from 2011 to 2017, particularly for the 3 age groups between 35 and 49 (decrease of 13.4%-16.9%). One of the reasons for the high rate of PSA test use, even after excluding the tests for which PSA test is indicated (e.g. history of prostate cancer), might be that PSA test is being used as screening for prostate cancer. Further research is needed to understand why PSA tests are being ordered, particularly for younger men.

A-142

Unique Role for Pharmacists in Clinical Translational Care and Outcomes: A Clinical Laboratory Story from a Fee-for Service to Value-Based Health Care Model

M. Dodd, K. Swanson, T. Borunda, D. Grenache. *TriCore, Albuquerque, NM*

As of January 1, 2018, changing payment rates for clinical laboratory tests, as a result of the Protecting Access to Medicare Act of 2014, is rendering a fee-for-service business model not sustainable. In response to the Centers for Medicare & Medicaid Services new payment system, it is necessary for clinical laboratories to innovate a new business strategy for delivering value-based care. Recognizing the unique skill sets of clinical pharmacists, TriCore Reference Laboratories has positioned these health professionals at the forefront of developing targeted interventions for high-cost, high-risk, and high-frequency healthcare conditions. Together with a data analytics team, the targeted interventions translate laboratory data into pre- and post-analytical insights with the goal to impact population health and improve outcomes. For example, capitalizing on the ability of the clinical laboratory to retrieve critical information upon test order through ask-at-order entry questions, a required pre-test probability score (4T score) was implemented for suspected heparin-induced thrombocytopenia. The objective was to reduce unnecessary testing and support treatment decisions with expensive alternative therapies. After implementation with an integrated health system, testing for a low 4T score between 0-3 decreased from 81% to 15% with a cost savings of $58,000 annually. Additionally, an automated post-analytical interpretive report for chronic opioid management was developed to offer clinicians a concise interpretation of the opioid testing results. The report identifies consistent and inconsistent results for definitive opioid testing based on the patient's prescribed opioids provided with the test order. The limitation of medical record interoperability across New Mexico hospitals has initiated investigation into insights from longitudinal microbiology data. From this data, pharmacists collaborated with the clinical microbiology medical director to develop an alert for emergency department clinicians. The alert would identify patients with a history of specific multi-drug resistant organisms at admission and prompt early isolation precautions. Pharmacists are uniquely trained in clinical and therapeutic medicine. They are recognized as integral members of the health care team in both the hospital and clinic setting. The clinical laboratory creates

a new environment for pharmacists to collaborate with laboratorians on the creation of targeted interventions for clinical decision support at the pre- and post-analytical phases of laboratory testing. TriCore is restructuring its business strategy from a tertiary contributor to an essential component of a value-based health care model in the state of New Mexico by incorporating pharmacists into their laboratory team.

A-143

Plasma Metabolites to Predict Response to Exercise in Alzheimer's Disease

G. Altawallbeh, M. Shyne, D. M. Vock, M. Y. Tsai, L. Li, F. Yu, D. Li. *University of Minnesota, Minneapolis, MN*

Background: Exercise intervention is a promising therapy for individuals with Alzheimer's disease (AD); meanwhile, plasma metabolites such as plasma phospholipids and amino acids have been associated with cognitive performance in older adults. Therefore, exercise and plasma metabolites may have synergistic effects on improving cognition in older adults with AD. The objective of this study was to identify plasma metabolites that interact with exercise and predict cognitive changes. **Methods:** This study was built on the FIT-AD Trial, a pilot randomized controlled trial to investigate the effects of a 6-month, individualized, moderate-intensity cycling intervention (20-50 minutes per session, 3 times a week) on cognition in community-dwelling older adults with mild-to-moderate AD (www.clinicaltrails.gov registration #: NCT1954550). FIT-AD subjects were randomly assigned on 2:1 ratio to cycling and control (sham exercise). Fasting blood plasma samples were collected at baseline (n=26). Targeted metabolomics analysis, by means of the commercial Biocrates p180 kits, was used to measure the levels of 188 metabolites including phospholipids and amino acids. Cognition was assessed at study baseline and 12 months using the Alzheimer's Disease Assessment Scale-Cognition (ADAS-Cog). Multiple linear-regression models (with 12-month change in cognition as the outcome and baseline plasma metabolite levels [log-transformed], group allocation, and their interaction as predictors) were used to identify metabolites whose interaction with exercise were associated with 12-month cognitive changes. Separate models were fit for each metabolite and adjusted for age and sex. Bonferroni adjusted p value of less than 0.0004 was used to indicate statistical significance; a p value between 0.0004 and 0.05 (including 0.05) indicates a trend of association. **Results:** Two phospholipids (lysoPC a C18.2 and PC aa C40.6) and 5 amino acids (isoleucine, leucine, methionine, tyrosine, and valine) interacted with exercise treatment and were associated with 12-month change in cognition as follows -27.992 (0.048), 14.447 (0.035), 28.372 (0.006), 26.787 (0.021), 32.436 (0.047), 33.747 (0.001), and 27.409 (0.023) for beta (p-value), respectively. All of them had positive interactions, which meant higher levels of the metabolites would predict better cognitive responses to exercise in AD, except lysoPC a C18.2, for which lower levels associated with better cognitive response. **Conclusion:** This study identified potential plasma metabolites that may have synergistic interaction with exercise in cognitive response to aerobic exercise. A future follow-up study with a bigger sample size is necessary to establish the ability of these metabolites to predict cognitive responses to aerobic exercise treatment in AD.

Tuesday, August 6, 2019

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

Proteomics and Metabolomics

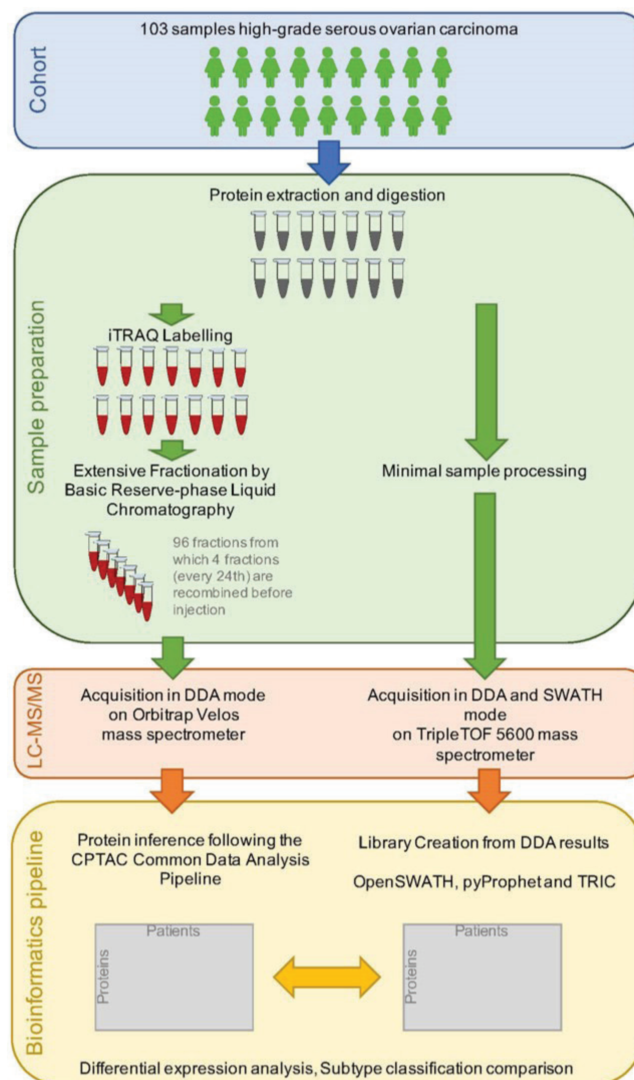
A-144

Enhanced Efficiency of Large-scale Clinical Proteomic Studies Using Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH-MS)

S. Thomas[1], B. Friedrich[2], M. Schnaubelt[1], R. Aebersold[3], D. Chan[1], H. Zhang[1]. [1]*Johns Hopkins University, Baltimore, MD,* [2]*Institute of Molecular Systems Biology, ETH Zürich, Zürich, Switzerland,* [3]*ETH Zürich and University of Zürich, Zürich, Switzerland*

Background: Seminal studies applying proteogenomic approaches to cancer tissue classification have been conducted, including the comprehensive proteomic characterization of high-grade serous ovarian cancer (HGSOC) tumors by the National Cancer Institute Clinical Proteomic Tumor Analysis Consortium (NCI CPTAC) [PMID: 27372738]. However, these large-scale clinical proteomic studies entail complex workflows and require extensive resources. In this study we applied an emerging, robust, high-throughput and quantitatively accurate proteomic technology, SWATH/DIA mass spectrometry, on aliquots of the same samples that were used in the original ovarian cancer CPTAC study. We compared the SWATH workflow with the more widely-used stable isotope labeling combined with 2DLC-MS/MS (iTRAQ DDA) workflow on the basis of cost, robustness, complexity, ability to detect differential protein expression, and the elucidated biological information.

Methods:



Results: Despite the greater than 2-fold difference in the analytical depth of iTRAQ DDA compared to SWATH-MS, common differentially expressed proteins in enriched pathways associated with the HGSOC Mesenchymal subtype were identified by both workflows with 96% of the proteins quantified by SWATH-MS also being quantified by iTRAQ DDA. Tumor subtype classification stability was sensitive to the number of analyzed samples, and the statistically stable subgroups were identified by the data from both methods. Additionally, the homologous recombination deficiency (HRD)-associated enriched DNA repair and chromosome organization pathways were conserved in both data sets.

Conclusion: SWATH-MS is a robust proteomic method that can be used to elucidate cancer-associated disease mechanisms resulting from the analysis of tumor tissue. The lower number of proteins detected by SWATH-MS compared to the iTRAQ DDA workflow is mitigated by the streamlined and less complex workflow, increased sample throughput (requiring ~80% less time), ~10-fold reduced sample requirements and 3-4-fold reduced cost. SWATH-MS therefore presents novel opportunities to enhance the efficiency of clinical proteomic studies and to potentially accelerate the application of this powerful proteomic approach to cancer biology.

A-145

Application of Platelet Bioenergetics and Metabolomics for Precision Medicine

B. K. Chacko[1], M. R. Smith[2], M. S. Johnson[1], G. A. Benavides[1], M. L. Culp[1], R. W. Hardy[1], K. Uppal[2], Y. Go[2], D. P. Jones[2], V. M. Darley-Usmar[1]. *[1]University of Alabama at Birmingham, Birmingham, AL, [2]Emory University, Atlanta, GA*

Background: Mitochondrial function and metabolism are increasingly being recognized as unique tools for developing precision medicine strategies for clinical applications. Recent studies show alterations in platelet cellular bioenergetics in diseases such as diabetes mellitus, sepsis and neurodegenerative diseases, which is proposed to be mediated by mitochondrial dysfunction and metabolism. The impact of mitochondrial dysfunction on cellular responses is determined by epigenetics, age, environmental factors and inflammation. The influence of all these factors create heterogeneity in individual bioenergetic responses and in the metabolite signature of platelets which highlight a potential opportunity for developing a precision medicine based-strategy by combining mitochondrial function and metabolomics. Using a multifactorial approach involving bioinformatics, metabolomics and cellular bioenergetics, we are demonstrating the interaction between cellular metabolites and mitochondrial function in human platelets. **Methods:** Platelets were either isolated from healthy subjects or collected from platelet bags from the Blood Bank, University of Alabama at Birmingham. Exclusion criteria for healthy subjects (85) are active diseases, recent surgical procedures, medications, supplements, smoking, pregnancy etc. Mitochondrial function was measured using Extracellular Flux Analysis and the untargeted metabolomics was determined by high-resolution mass spectrometry. Association studies determining the relationships of the bioenergetic parameters with specific metabolites were performed using the bioinformatics tool xMWAS. **Results:** Multivariate correlation studies using 85 healthy donor samples showed positive correlation of ATP-linked oxygen consumption rate (OCR) with basal and maximal OCR but a weak relationship with proton leak. Reserve capacity was positively correlated only with maximal respiration. Mitochondrial Complex activities were not strongly correlated with each other with the exception of Complex II/ FCCP vs Complex II/ ADP and a weak association of Complex I and IV. The respiratory control ratio was positively correlated with ATP synthase activity. Mass spectrometry analysis (of the 13 platelet samples collected from the blood bank) identified over 3,500 metabolic features of which 3,150 were present in 8 out of 13 donors. Analysis of the 3150 features using the KEGG pathway database show that 924 features representing 58 metabolic pathways including arachidonic acid metabolism, glycolysis and fatty acid metabolism, which are critical for platelet function. Interestingly, the unsupervised metabolome heat map for the 11/13 donors showed two distinct metabolic clusters in the vehicle group which differed in 12 metabolic pathways. The result of xMWAS analysis yielded 4 distinct communities encompassing over 100 features and 6 bioenergetic parameters with the number of positive and negative associations between metabolites and bioenergetics parameters ($r > 0.5$, $p < 0.05$). Communities 1 and 2 have the strongest number of interactions with Reserve Capacity and Maximal OCR, whereas community 4 has a stronger influence over AL and Basal OCR. Community 3 has the smallest number of interactions and is associated with non-mitochondrial OCR and proton-leak. **Conclusions:** This study demonstrates the unique relationships between bioenergetic parameters in healthy subjects, which form a bioenergetic program. The data also demonstrate correlations between bioenergetic parameters with almost 200 metabolites in the resting platelet. Targeting of these relationships has the potential of mapping mechanisms of diseases and profiling patients for precision medicine strategies.

A-146

A Metabolomics Approach to Identify Plasma Biomarkers for Alzheimers Disease

C. Lin, C. Huang, K. Huang, K. Lin, T. Yen, H. Kuo. *Chang Gung Memorial Hospital, Taoyuan, Taiwan*

Background: Alzheimer's disease (AD) is a progressive degenerative disorder of the brain and is the most common form of dementia. AD currently affects over 35 million individuals; death occurs on average nine years after diagnosis. A useful screening is essential to identify cognitive normal individuals in old aged who have high risk of developing AD. Brain imaging and the analysis of cerebrospinal fluid samples are important but expensive and invasive for verifying the diagnosis. Blood metabolites levels represent the dynamic metabolomics status of living system. The aim of our study was to identify blood metabolites with altered levels of expression in patients with early and progressive stages of AD. **Methods:** All participants of the study underwent genetic screening and were accurately diagnosed with the combination of neuropsychological assessment and amyloid imaging prior to metabolomics analysis. The Clinical Dementia Rating Scale (CDR) was used to rate the dementia severity; including CDR=0 (as normal group), CDR=0.5 (as mild cognitive impairment, MCI group) and CDR≥1 (as Alzheimer disease group, AD group). A total of 40 participants were included in this study, including 15 normal, 10 MCI and 15 AD. Plasma samples were collected and applied to liquid chromatography MS/MS assay based metabolomics analysis. **Results:** Our results showed that the plasma levels of C3, C5 and C5-DC acylcarnitines, arginine, phenylalanine, creatinine, symmetric dimethylarginine (SDMA) and phosphatidilcholine PC ae C38:2 were significant altered in patients with early and progressive stages of AD. Using a machine learning approach, we created a predictive model based on the decision tree that included three main parameters: age, arginine and C5 concentrations. The model distinguished AD patients from other participants with 60% sensitivity and 86.7% specificity. For healthy controls sensitivity was 85.7% and specificity was 61.5%. Multivariate ROC analysis performed for decision tree showed that our model reached an acceptable diagnostic power in differentiating cognitively normal aged people (AUC=0.77) and those with AD (AUC = 0.72).

A-147

Analytical Accuracy of a Urine Metabolite Panel Using NMR Spectroscopy Developed for Identification of Kidney Allograft Rejection

R. J. Scott, B. E. Burns, A. S. Jaffe, J. C. Lieske, J. W. Meeusen. *Mayo Clinic, Rochester, MN*

Background: Improving long-term kidney transplant survival is a pressing public health need given the demand for and availability of donor kidneys. An untargeted urine metabolomics model was recently developed that reported an AUC of 0.78 for identifying acute rejection in a cohort of kidney transplant recipients between 2 weeks and 1 year after transplant. Metabolomic methods often are useful in biomarker identification, but at times lack the analytical performance necessary for routine clinical testing. The objective of this study was to validate the analytical performance of this nuclear magnetic resonance (NMR) spectroscopy-based urine metabolomics method in anticipation of evaluating its use for detecting acute rejection. **Methods:** Urine alanine, citrate, creatinine, lactate and urea were quantified using a Bruker Ascend 600 NMR (Bruker, Billerica, MA) with AXINON software (numares AG, Regensberg, Germany). Accuracy was assessed by comparing NMR results to conventional chemistry methods (n=50). Alanine was quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS); and citrate, creatinine and urea were analyzed on a Roche Cobas c501 (Roche, Indianapolis, IN). No established clinical laboratory method for urine lactate was available. Stability was assessed in urine from 10 subjects by comparison to results at time zero. Precision and limit of quantification (LoQ) were assessed by duplicate measures twice daily for 20 days. Linearity was assessed by mixing urine with high and low concentrations at various ratios and measuring in triplicate. **Results:** Bias between NMR and conventional methods was <20% with R^2 >0.93 in all cases (Table). Ambient stability is limited to 8 hours (due to lactate); all analytes were stable 14 days at 4ºC and 30 days frozen. **Conclusion:** The analytical performance of this NMR-based urine metabolite panel is equivalent or better than that of conventional chemistry methods for these analytes. With sufficient qualification, NMR-based metabolomic methods are suitable for clinical use.

A-148

Protein Assisted Digestion Improves Sensitivity of Immunocapture-MRM Method to Quantify Stool Biomarker of Colorectal Cancer

R. Bearden, R. Alagandula, B. Guo. *Cleveland State University, Cleveland, OH*

BACKGROUND: Quantitative LC-MS/MS analysis of protein biomarkers is a major challenge in clinical research due to the low abundance of most biomarkers. The most critical step in sample preparation for bottom-up proteomics is the conversion of proteins to peptides. Digestion has always been a major obstacle in developing a truly quantitative method where there are low quantities of protein. The physiological concentrations of most protein biomarkers are sub-picomolar and while current mass spectrometers have detection capabilities at low femtomolar ranges, the sensitivity for identification of proteins after gel-separation or immunocapture and digestion is far less than the sensitivity of current mass spectrometers due to inefficient digestion. In this research we aimed to develop a more sensitive immuno-affinity mass spectrometry-based method to quantify hemoglobin, a stool biomarker of colorectal cancer by improving the enzymatic digestion of small quantities of protein. Lower

limits of quantification would address the need to reach sensitivities lower than current fecal immunochemical tests (FIT) for hemoglobin in stool so that cut-off values can be chosen to better predict risk.

METHODS: Digestion was performed in-well on the captured protein, hemoglobin. Bovine serum albumin (BSA) was spiked into the solution prior to the addition of trypsin. Two peptides from alpha and beta subunits (MFLSFPTTK and EFTPPVQAAYQK) and their stable isotope-labeled standards were used for accurate quantification by LC-MS/MS analysis. Quantitative yield was based on signal intensity, peak area, and number of peptides rather than qualitative measures such as %SQ and missed cleavages. Peak areas of quantitative alpha (*m/z* $536.3^{++} \rightarrow 593.3$) and beta (*m/z* $690.1^{++} \rightarrow 807.5$) peptides were used to assess the effect of adding BSA on digestion efficiency compared to digestion without BSA.

RESULTS: Digestion was evaluated at the LLOQ (5 ng) and around the recommended concentration of protein for in-solution digestion. Without the addition of BSA, peak areas at the LLOQ averaged 513 and 373 cps, and with the addition of 2 ng BSA just prior to adding trypsin, the average peak area increased to 1.9×10^4 and 1.1×10^4 cps, which was comparable to peak areas for hemoglobin a thousand times more concentrated: 6.0×10^4 (α MFLSFPTTK) and 4.5×10^4 (β EFTPPVQAAYQK). Digestion in the presence of BSA produced more intense signals lowering the LOD from 5 ng to 1.0 ng and LLOQ from 20 ng to 5 ng with a S/N threshold above 5, thereby increasing confidence in quantification, making this affinity-MS assay an order of magnitude more sensitive than commercial FITs with microgram cut-off values. There was no interference from BSA in any of the MRM channels demonstrating that the presence of BSA enhanced quantification of the target protein at low concentrations.

CONCLUSION: After achieving quantitative digestion of peptides, the LLOQ was lowered 4-fold by the addition of BSA before enzymatic digestion in what we refer to for the first time as protein assisted digestion. We demonstrated that the addition of BSA increases digestion efficiency for small quantities of protein and therefore making quantification of femtomolar range of hemoglobin and thus many protein biomarkers a possibility.

A-149

The Lumipulse® G whole Parathyroid Hormone (wPTH) Assay: A Fully Automated Method for Quantitative Determination of wPTH in Human Serum and Plasma

N. Benina[1], K. Falcone[1], S. Raju[1], S. Marcoon[1], K. Le[1], M. Wang[1], N. Van der Cruyssen[2], M. Vandijck[2], B. De Decker[2], A. Rombout[2], J. Genzen[3], D. Grenache[3], J. Hunsaker[3], G. Lambert-Messerlian[4], E. Eklund[4], K. Donaldson[5], P. Wheeler[5], R. Radwan[1], S. Dolan[1], D. Dickson[1], C. J. Traynham[1]. *[1]Fujirebio Diagnostics Inc, Malvern, PA, [2]Fujirebio Europe, Ghent, Belgium, [3]ARUP, Salt Lake City, UT, [4]WIHRI, Providence, RI, [5]Prescient Medicine, Hummelstown, PA*

Introduction: Parathyroid hormone (PTH) is secreted from the parathyroid glands as an 84 amino acid polypeptide and regulates metabolism of calcium and phosphoric acid. Higher internal accumulation of PTH can be seen in patients with renal diseases including chronic renal dysfunction reflecting a metabolic disorder of PTH in the kidney. Therefore, *in vitro* measurement of bioactivated PTH (1-84) in blood can be used to determine function of the parathyroid glands and several types of calcium-metabolic bone diseases. **Methods:** The Lumipulse G whole PTH (wPTH) assay is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the *in vitro* quantitative determination of wPTH in human serum and plasma on the LUMIPULSE G System. wPTH specifically binds to alkaline phosphatase labeled (ALP; calf) anti-PTH polyclonal antibody (goat) coated on particles and forms antigen-antibody immunocomplexes. After washing, the amount of wPTH is derived from the luminescence signals generated by adding substrate solution containing AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt). All verification and validation studies were performed according to their respective CLSI guidelines. **Results:** The Limit of Blank, Limit of Detection and Limit of Quantitation of the Lumipulse G wPTH assay were 0.0 pg/mL, 0.3 pg/mL, and 2.1 pg/mL, respectively. The Lumipulse G wPTH assay demonstrated linearity in the range from 1.4 to 2190.3 pg/mL. No high-dose hook effect was observed in samples containing >60,000 pg/mL of wPTH. A twenty-day precision study of 5 human serum-based panels (n = 30 for each panel) was tested in triplicate, and demonstrated total precision of ≤ 6.7%. Spike-recovery testing showed the Lumipulse G wPTH assay can recover known concentrations of supplemental analyte. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 9 endogenous substances (cholesterol, albumin, free bilirubin, conjugated bilirubin, triglycerides, human anti-mouse antibodies, rheumatoid factor, chyle and hemoglobin) and 19 commonly used therapeutic drugs. Cross- reactivity of Lumipulse G wPTH assay with other substances (Calcitonin (500,000 pg/mL), Osteocalcin (500,000 pg/mL), c-telopeptide (500,000 pg/mL) and PTH 7-84, 1-34, 39-84,39-68,44-68, 53-84, and 13-34 (200,000 pg/mL), respectively) that are similar in structure to wPTH demonstrated no cross-reactivity. EDTA anticoagulants K2EDTA and 2NA EDTA negatively impacted assay results (>10% difference) at concentrations above 2 mg/mL. Lumipulse G wPTH immuno-cartridges and calibrators were shown to be stable at intended storage conditions (2-10°C) for up to 7 months. A sample stability study revealed serum and plasma specimens can be stored at -20 for up to 3 months, while refrigerated serum and plasma can be refrigerated for up to 24 hours and 7 days, respectively. A freeze-thaw study revealed no more than 1 freeze-thaws should be performed in samples collected in the following tubes: Red top serum, high speed clotting tube, SST serum, K_2EDTA plasma, sodium heparin plasma and Lithium Heparin plasma tubes. **Conclusions:** The data demonstrate that the Lumipulse G wPTH assay on the automated LUMIPULSE G1200 System is sensitive for routine quantitative determination of wPTH in serum specimens.

Tuesday, August 6, 2019

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

Endocrinology

A-150

Decreased Serum Glucose-6-Phosphate Dehydrogenase Level is Related to Increased Risk of Diabetes and Renal Impairment

N. Basnet[1], K. Gautam[2], S. Pradhan[2]. [1]*Sumeru Hospital, Lalitpur, Nepal,* [2]*Samyak Diagnostic Pvt. Ltd., Lalitpur, Nepal*

Background: Diabetes and its complications including renal impairment have been suggested to be consequences of increased oxidative stress. Glucose-6-phosphate dehydrogenase (G6PD) is an enzyme of pentose monophosphate shunt which is responsible for protection from harmful oxidative metabolites. G6PD deficiency, the most common enzyme deficiency in human, leaves these individuals at high risk of oxidative stress and at high risk of diabetes and its complications including nephropathy. The relationship between G6PD deficiency and diabetes is still a topic of discussion. Whether deficiency of G6PD leads to diabetes or raised blood glucose leads to G6PD deficiency has also been debated and both views are supported by experimental studies. This study was carried out to determine the relationship between G6PD deficiency state in the diabetic population and the presence of nephropathy in these patients.

Methods: For the study, 68 consenting volunteers were recruited. Healthy control volunteers and diabetic with or without renal impairment were selected after counseling when they came for their regular follow-up. Those who were unwell, admitted in hospital, or on renal replacement therapy were excluded from the study. A detailed history of drugs, diabetes and other co-morbidities, and tobacco and alcohol use were noted. Body Mass Index and blood pressure were recorded. Blood tests to measure the G6PD activity in fresh red blood cells, hemoglobin, glycated hemoglobin, uric acid, and creatinine were done. Urine was tested for the presence of proteinuria. The activity of G6PD was measured in U/dl and categorized as normal (>50 U/dl), mild to moderate deficiency (30-49 U/dl), and severe deficiency (<30U/dl). Glomerular filtration rate (eGFR) was estimated with the Modification of Diet in Renal Disease Study (MDRD) Equation.

Results: Out of the 68 volunteers, 27 were female and 41 were male with mean age of 49.95 +/- 1.9 years. Twenty four were healthy controls who did not have history of any kidney disease. The remaining 44 were diabetic with duration of from 2 months to 31 years (mean: 9.45 +/- 1.38 years). The level of hemoglobin was within normal range in controls; and 13 patients with diabetes had hemoglobin less than 10 mg/dl. All the individuals in control group had a normal renal function as estimated by MDRD equation. Diabetic patient with normal renal function had average eGFR 102.6 +/- 8.9 ml/min/1.73 m^2 with eight having microalbuminuria. Diabetic volunteers with renal impairment had average eGFR of 26.7 +/- 3.72 ml/min/1.73 m^2 and microalbuminuria. 12.5% of healthy controls had a low level of G6PD activity whereas 26.32% and 40% of diabetic patients without and with renal impairment had various degree of G6PD deficiency. Moreover, the mean G6PD level among diabetic patients (60.3 U/dl) was significantly lower than that of healthy control (78.37U/dl). The G6PD activity level was lowest for diabetic patients with renal impairment (51.32 U/dl).

Conclusion: The prevalence of diabetes patients is in increasing trend and many of them ultimately develop renal complications. This study supports the association between decreased G6PD with diabetes and diabetic nephropathy.

A-152

Effects of Short-Term and Long-term Use of Lithium on Thyroid Function Tests in Patients with Bipolar Disorder

R. Gupta[1], A. Bhattarai[2], M. Raut[2], V. Sharma[2], B. Yadav[2], B. Jha[2]. [1]*National Public Health Laboratory, Kathmandu, Nepal,* [2]*Maharajgunj Medical College, Institute of Medicine, Kathmandu, Nepal*

Background: Lithium has been used by most psychiatrists as a long term effective therapy for the treatment of bipolar disorder as well as reducing the risk of suicide and short term mortality in patients. However, some studies have also reported varying degrees of thyroid abnormalities in lithium treated patients, but it is unclear whether there is significant association with duration of therapy. We aimed to determine the effect of long term use of lithium on thyroid function tests and possible prevalence of hypothyroidism in women and men with bipolar disorder.

Methods: This cross-sectional study was conducted in 75 bipolar disorder patients (24 males, 51 females) treated with lithium and equal number of controls. Diagnosis of bipolar disorder was made by psychiatrist according to ICD-10-DCR guidelines and DSM-IV criteria. Serum fT3, fT4 and TSH were measured by enhanced chemiluminescence immunoassay. Statistical analysis was performed using SPSS 20.0 version.

Results: The prevalence of primary hypothyroidism and subclinical hypothyroidism were found significantly increased in lithium treated group (12% and 17% respectively) which were further increased with duration of treatment, showing no significant difference of subclinical hypothyroidism in sex (17.6% female vs. 16.6% male), but primary hypothyroidism cases were only observed in female (17.6%) not in male. The mean fT3 level of lithium treated group was decreased as compared to control group (5.61±1.35 vs. 6.02±1.1, p= 0.051), also showing decreased level of fT4 (17.57±6.35 vs. 19.71±4.56, p = 0.019). But mean TSH level was found significantly (P lt 0.001) higher in lithium treated group than that of control (9.67±12.47 vs. 3.41±3.69).

Conclusion: Our findings indicate that use of lithium therapy is associated with higher degree of primary hypothyroidism and subclinical hypothyroidism which is being increased with duration of lithium therapy. These results also show slightly higher prevalence of hypothyroidism in female but statistically not significant.

A-153

Circulating miR-421 Expression is Associated with Insulin Resistance in Metabolic Syndrome Patients

R. H. Bortolin[1], A. A. Braga[1], E. M. G. Saldarriaga[1], T. D. C. Hirata[1], A. Cerda[2], R. C. C. Freitas[1], H. L. Wang[3], J. B. Borges[3], J. Í. D. França[3], L. N. Masi[4], R. Curi[4], T. P. Curi[4], M. F. Sampaio[3], L. R. Castro[3], G. M. Bastos[3], R. D. C. Hirata[1], M. H. Hirata[1]. [1]*University of Sao Paulo, Sao Paulo, Brazil,* [2]*Universidad de La Frontera, Temuco, Chile,* [3]*Dante Pazzanese Institute of Cardiology, Sao Paulo, Brazil,* [4]*Cruzeiro do Sul University, Sao Paulo, Brazil*

Background: Epigenetics play important roles in the regulation of genes involved in metabolic diseases and other pathophysiological conditions and circulating micoRNAs (miRNAs) may contribute to the identification of novel biomarkers and/or drug targets. We investigated the expression of circulating miRNAs in patients with metabolic syndrome (MetS) and the association of the differentially expressed miRNAs with components of MetS and inflammatory biomarkers. **Methods:** A sample of Brazilian patients were classified in MetS (n=88) and non-MetS (n=144) groups according to the International Diabetes Federation criteria. Blood samples were obtained for clinical laboratory testing and miRNA expression analysis. **Results:** The results of anthropometric measures, serum lipids, glycemic control and inflammatory biomarkers showed an increased cardiometabolic risk profile in MetS group compared to non-MetS group (p<0.05). Insulin resistance (IR) was more frequent in MetS patients (85.4%) compared to non-MetS (32.6%, p<0.001). Screening of serum-derived miRNAs (372 targets) was carried out using miRNA PCR array in a small group of MetS patients (n=6) and healthy controls (n=6). The result showed miR-183-5p and miR-301a-3p had lower expression and miR-542-5p, miR-424-3p, miR-326, miR-421 and miR-574-3p were overexpressed in MetS subjects (p<0.05; FC>2) compared with health subjects. Analysis of the differentially expressed miRNAs in the total group showed that serum miR-421 remained upregulated in MetS group in comparison to non-MetS group (p<0.05). Serum miR-421 expression was positively correlated with Hb1Ac (r= 0.151, p=0.023), triglycerides (r= 0.148, p=0.025), VLDL-c (r= 0.148, p=0.025), hs-CRP (r= 0.145, p=0.030), IL-6 (r= 0.186, p=0.006) and resistin (r= 0.146, p=0.030), and inversely correlated with adiponectin (r= -0.138, p=0.041). A stepwise multiple logistic regression analysis showed that upregulation of serum miR-421 was associated with increased risk for MetS (OR=1.15, 95%CI=1.01-1.32, p=0.046), hypertension (OR=1.12, 12%; 95%CI=1.01-1.26, p=0.045) and IR (OR=1.21, 95%CI=1.04-1.41, p=0.012). Increased leptin was associated with increased risk for IR (OR=1.15, 95%CI=1.09-1.22, p<0.001). miR-421 expression was also higher in IR patients compared to non-IR group (p=0.014). Predictive bioinformatics analysis demonstrated that miR-421 targets 11 mRNAs (*ADIPOQ, PLA2G1B, IL2, MAPK14, MAP2K3, PTPN11, CASP3, ACSL4, PRKAA2, AKT3 and CEBPB*) involved in regulatory pathways related to type 2 diabetes and/or glycemic changes, obesity, liver inflammation and steatosis and inflammatory response. **Conclusion:** The results are suggestive that circulating miR-421 may represent a new potential biomarker for IR and other components of cardiometabolic risk in MetS patients.

A-154

Assessment of the Relation between Body Fat Composition and Serum Kisspeptin Level in Obese versus Non Obese Women at the Time of Ovulation in Egypt

S. H. Hassan, N. M. El Azhary, N. S. El beltagy, N. K. Mohamed. *Alexandria University, Alexandria, Egypt*

Background: Kisspeptin (previously known as metastatin) plays a role in the metabolic regulation of fertility and the generation of cyclic LH surge that precedes ovulation. Recently, the effects of lifestyle and dietary fat intake on female reproductive health have received new attention. In fact, obese women are at an increased risk of developing infertility. No previous study has assessed the relation between body fat composition and kisspeptin level in the prediction of ovulation in infertile women due to [poly cystic ovarian syndrome(PCOS). This is the first time to measure and evaluate the relation between body fat composition and the serum kisspeptin level at the time of ovulation among obese and normal weight infertile women and their matched control in Egypt. **Method:** a case control study was done in the department of obstetrics and gynecology of Alexandria medical school between February and October 2017. Forty infertile women with PCOS (20 obese women with BM Imore than or equal to30 kg/ m ^{2} and 20 women with BMI less than 30 kg/ m ^{2}) were matched for age and BMI with twenty healthy control. All women participated in food frequency questionnaire for dietary habits. Body fat composition in term of subcutaneous adipose tissue thickness determined by skin fold caliper and body composition analyzed by inbody apparatus. Blood samples were obtained at day 11 of the cycle to determine serum kisspeptin by ELISA kit in the physiology laboratory of endocrinology in Alexandria medical school. Difference between means was analyzed using ANOVA. Pearson's correlation was also used for analysis. **Results:** serum kisspeptin level was significantly lower among infertile PCOS obese women than the infertile women with BMI less than 30 kg/ m ^{2} and the control group (p=0.04). Waist to hip ratio was significantly higher among the obese infertile women compared to the other two groups (p=0.01). There was negative correlation between serum kisspeptin and both body fat percentage and BMI (R =-0.70, R= -0.59) respectively. **Conclusion:** because serum kisspeptin is strongly correlated with body fat composition, there is a need to use body fat composition instead of BMI in screening of infertility cases due to PCOS

A-155

Genetic Analysis of *RET proto-oncogene* in Suspected Carriers of Multiple Endocrine Neoplasia Type 2 Born in Argentina

M. Viale, M. Serra, T. Fernandez-Gianotti, A. Kozak, E. Miler, G. Rubino, A. Stigliano, R. Garraza, M. Russo Picasso, P. Fainstein Day. *Hospital Italiano de Buenos Aires, CABA, Argentina*

Multiple endocrine neoplasia type 2 (MEN 2), is an autosomal dominant cancer syndrome caused by missense gain-of-function mutations in the RET proto-oncogene, on chromosome 10. There are two different clinical variants of MEN2: MEN2A, which includes the familial medullary thyroid cancer subtype (FMTC), and MEN2B. MEN2A show a penetrance higher than 90% medullary thyroid carcinoma (MTC), 50% pheochromocytoma and 25% primary hyperparathyroidism. MEN2 B accounts for 5% of hereditary MTCs and comprises MTC, pheochromocytoma and ganglioneuromatosis. FMTC is characterized by presenting only MTC in a kindred with a minimum of 4 family members. Genetic testing for the RET proto-oncogene in possible carriers and in their first-degree relatives allow to identify mutations in 98% to 100% of evaluated cases. It requires the sequencing of the most frequent variants, which are usually located in the exons 10,11,13,14,15 and 16 and are call hot spot zones. Nowadays there are also mutations reported in exon 5, 8 and 18. There is strong genotype-phenotype correlation and RET mutations classified into three prognostic risk levels: moderate, high, and highest-risk, determined by age of onset and the potential aggressiveness of MTC. Thus, the management of these patients is determined by the result of the genetic testing. Prophylactic or early thyroidectomy in patients with a germline *RET* mutation characteristic of hereditary MTC has become the standard of care worldwide as it can prevent or cure MTC in MEN2. **Aim:** To evaluate the RET proto-oncogene germline mutations in patients with clinical features of MEN 2 born in Argentina. **Subjects:** We studied 79 potential carriers: 70 were index-cases and 9 first-degree relatives. All subjects gave written informed consent. **Methods:** Genomic DNA was obtained from peripheral blood leukocytes. Coding region of exons: 10, 11, 13, 14, 15, 16 and later 5, 8, 18 and intronic flanking regions were amplified by PCR. The DNA fragments were sequenced after being manually labeled with ddNTP33 from 2002 to 2008, and by automatic Sanger Sequencing since 2009. Pathogenic variants were confirmed in another DNA sample. **Results:** We found germline mutations in 10% (numero; yo en realidad prefiero el numero de casos en el texto y el porcentaje entre parentesis) of index-cases and in the 55.6% (numero)of the first-degree relatives. The germline variants found /Exon /Phenotype were: c.2753T>C/16/MEN 2B; p.Cys611Trp/10/FMTC-MEN2A; c.1902C>G/11/ MEN2A; c.2304G>C/13/ FMCT; c.2410G>A/14/MEN2A-FMTC. All mutations in index cases were missense mutatons, and were clinically associated with MEN2A, MEN2B, and FMTC. The SNPs found were (germinal variant/ exon/ protein variant): c.2307T>G/13/p.L769L; c.2071G>A/11/p.G691S; c.2712C>G/15/p.S904S; c.2508C>T/14/p.S836S; c.2392+312G>A/intron13. **Conclusions:** Germline mutations were detected in 10 % of MEN 2 index cases and 55.6% of the first-degree relatives. They were located at the hot spot zones and showed correlation between phenotype and genotype, as observed in other published series.The specific germline activating mutation of the RET proto-oncogene appears to be the main determinant of the age of MTC onset. Prophylactic thyroidectomy in carriers has changed the natural history of the disease, constituting the most representative case of primary prevention of cancer by genetic testing.

A-156

Invasive Venous Sampling in the Diagnosis of Endocrine Disorders - A Single Center Experience from Sri Lanka

U. D. Senarathne[1], S. K. A. D. Kariyawasam[2], B. K. T. P. Dayanath[3], E. Ganewatte[2], S. Siyambalapitiya[4]. *[1]Department of Biochemistry, University of Sri Jayewardenepura, Nugegoda, Sri Lanka, [2]Department of Radiology, Colombo North Teaching Hospital, Ragama, Sri Lanka, [3]Department of Chemical Pathology, Colombo North Teaching Hospital, Ragama, Sri Lanka, [4]Department of Endocrinology, Colombo North Teaching Hospital, Ragama, Sri Lanka*

Background: Interventional radiology assisted venous sampling (VS) can be labelled as the gold standard for localizing abnormal hormone secretion in few of the common endocrine disorders namely; primary hyperaldosteronism, pancreatic insulin secreting tumors, hyperparathyroidism, and adrenocorticotropic hormone dependent Cushing's syndrome. These endocrine tumors can cause significant morbidity and mortality among young, which merits proper diagnosis and pre-operative localization of the tumor for better management. This is mandatory as the management of the disease depends on the nature (unilateral/ bilateral) of the tumor with surgical resection being curative in many of the instances. Cross sectional radiological imaging alone is not conclusive to arrive at a diagnosis as the tumor activity cannot be assessed by imaging and most of these tumors are relatively small. In experienced hands with good liaison between endocrinology, radiology and chemical pathology; VS is a safe, accurate, and highly sensitive test for localizing such occult tumors and assessing their activity.

Methods: Fourteen patients were subjected to VS of adrenal (n=8), pancreatic (n=4) and parathyroid (n=2). Biochemical conformation of unequivocal hyperaldosteronism, hyperinsulinism, and hyperparathyroidism were done using serum aldosterone level, aldosterone: renin ratio (ARR) and failure to suppress aldosterone following saline infusion; plasma insulin level; and plasma PTH level respectively. For each VS, common femoral venous access was gained and selective catheterization of veins draining the areas of interested was performed. Adrenal venous sampling (AVS), arterial stimulated pancreatic venous sampling (ASVS), and parathyroid venous sampling (PTVS) were performed using standard procedures and sites. Selectivity (SI) and lateralization (LI) indexes were calculated for AVS (Rossi et al; 2014) and ≥2 taken as cut-off for both indices. In ASVS, ≥2 fold increase in insulin from basal within 60 seconds from calcium stimulation was taken as a positive response. The ratio of PTH levels in the specific vein: peripheral vein (PTHR) calculated in PTVS and ≥2 fold increase taken as a positive response.

Results: All eight patients who were selected for AVS has serum aldosterone >15ng/dL, ARR>30, with serum aldosterone >10ng/dL following saline infusion. Of them four patients had SI<2 on right side rendering the results unable to be used for lateralization of the lesion. The rest had mean SI of 10.5 on right, 8.7 on left with mean LI of 11.1 and mean contralateral suppression index of 0.3, enabling the diagnosis of unilateral (3 right and 1 left sided) adrenal tumors who were managed surgically with conformation of diagnosis.In three out of four patients who underwent ASVS, pancreatic lesions were localized with a mean insulin increase of 8.4 fold and managed surgically. The remaining patient demonstrated similar increase in insulin secretion from 4 out of 6 arterial territories and managed as Nesidioblastosis. Lesion was localized in both patients who underwent PTVS with mean highest PTHR of 4.3 folds and managed surgically.

Conclusion: VS is a highly sensitive investigation to localize and assess the tumor activity in adrenal, pancreatic and parathyroid tumors. The difficulty in selective catheterization poses a technical difficulty in its utilization in the clinical practice which could be overcome with experience.

A-157

Features of Metabolic Syndrome among Sri Lankan Women with Polycystic Ovary Syndrome - A Preliminary Study

U. D. Senarathne[1], B. K. T. P. Dayanath[2], S. Siyambalapitiya[3]. *[1]Department of Biochemistry, University of Sri Jayewardenepura, Nugegoda, Sri Lanka, [2]Department of Chemical Pathology, Colombo North Teaching Hospital, Ragama, Sri Lanka, [3]Department of Endocrinology, Colombo North Teaching Hospital, Ragama, Sri Lanka*

Background: Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorders of women in reproductive age group. It is associated with hyperandrogenism (HA), ovarian dysfunction (OD) and polycystic ovarian morphology (PCOM). Though not included in the diagnostic criteria, features of metabolic syndrome (MS) and insulin resistance (IR) are important associations of PCOS, which have become main research interests at present. Although likelihood of associated metabolic and cardiovascular risks related to PCOS has been investigated worldwide, it is yet to establish the underlying root cause of metabolic manifestations seen in PCOS.

Methods: Thirty five women fulfilling Rotterdam criteria were subjected to clinical, biochemical and ultrasound examination prior to initiation of any treatment to establish the diagnosis and assess IR (by Homeostatic model assessment of insulin resistance), hirsutism score (by Ferriman Gallway scale), acanthosis score (scale by Burke et al; 1999), and presence of MS (criteria by American Heart Association criteria). Biochemical parameters that were checked include; LH, FSH, testosterone, insulin, fasting plasma glucose, lipid profile which were assayed by immunoassay and spectrophotometric methods with acceptable assay performance.

Results: A majority presented with oligomenorrhoea (67%) with an average menstrual cycle length of 74 (SD=45.5) days at a mean age of 24 (SD=5.2) years. Fifteen (47%) of them fulfilled criteria for phenotype-A (HA, OD, PCOM) while the rest were included in phenotype-B (HA, OD). All of them had at least one first degree family member with a feature of MS. Their mean BMI was 25.75 kg/m^2 (SD=3.38) with 62.5% of them being overweight or obese. One third of the women (11) fulfilled diagnostic criteria for MS while all of them had at least one feature. MS score had a positive Pearson correlation with acanthosis score (p=0.008) and fasting insulin level (p=0.011) while women with MS had a significantly higher acanthosis score (p=0.002, t=1.526). Hirsutism score and serum testosterone level, plasma insulin level did not show any significant difference between women with and without MS (respective p values; 0.1, 0.7, 0.4). The mean IR among women with MS was >2.5 (2.55) but there was no significant difference between the 2 groups.

Conclusion: Features of metabolic syndrome are common among Sri Lankan women with PCOS. PCOS Women with MS had significant manifestations of insulin resistance like acanthosis nigricans though plasma insulin level was not significantly higher among them. HOMA-IR and Hyperandrogenism failed to show any statistically significant correlation to metabolic syndrome indicating a possible underlying genetic makeup for the manifestation of metabolic syndrome and manifestations of insulin resistance which merit further investigation into genetic makeup of the disease.

A-158

Improving Cortisol Measurement Turnaround Time in Adrenal Vein Sampling Studies

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

Background: Adrenal vein sampling (AVS) is the standard test to distinguish between unilateral adenoma and bilateral hyperplasia in the investigation of primary hyperaldosteronism. Rapid cortisol measurement allows clinicians to reposition the collection catheter and resample unsatisfactory samples, avoiding incomplete and non-diagnostic procedures. In early 2017, our laboratory was asked to set up a protocol to deliver rapid cortisol measurement on AVS samples. We describe our experience and the details of our workflow.

Methods: The biochemistry laboratory performs 4 million tests a year of which over 90% are run on the Beckman Coulter Power Processor laboratory automation system including 2 Beckman Coulter DxI-800 immunoassay analysers. Cortisol measurement is carried out on these analysers with an assay incubation time of 30 min and dilution required for results > 1600 nmol/L. The rapid cortisol workflow involved running undiluted, 10x and 20x diluted samples automatically for all AVS samples.

Results: The non-AVS cortisol median turnaround time from sample receipt to reporting was 93 min (undiluted) and 130 min (diluted) while for rapid cortisol samples the median was 88 min. 67% of rapid cortisol samples were > 1600 nmol/L and were reported from diluted sample testing.

Conclusion: Implementation of a rapid cortisol measurement pathway for AVS has resulted in a 42 min (32%) reduction in turnaround time for high (> 1600 nmol/L) cortisol samples. This has allowed a greater proportion of AVS studies to be satisfactorily completed in a single session, reducing the need for patient rescheduling for repeat studies.

A-159

Retrospective Study in Reference Intervals Verification to Thyrotropin Test in Pediatrics Population of Southern Brazil Using the *Advia Centaur XP Analyzer, Siemens*

D. Canali, D. Faria, A. Lopes, M. Soika, C. Fontes. *Laboratório Médico Santa Luzia, São José, Brazil*

Background: The aim of this study was to verify the thyrotropin (TSH) reference intervals (RI) of our database with similar studies that used the *ADVIA Centaur XP™ Analyzer, Siemens, USA,* as in specific guidelines (such as CALIPER reference interval database[1]; and Pediatric Reference Intervals edited by Soldin et al, 7th edition, AACCPress[2]), RI for this manufacturer are not available.

Methods: We included TSH results of the laboratory routine processed in the *ADVIA Centaur XP* outpatient patients between 0-18-year-old, without using medications for thyroid diseases and showing normal results for fT4, fT3, T4 and T3, processed between May 2016 and May 2017. The verification was compared with the studies of *Lim et al[3], Loh et al[4], Kahapola-Arachchige et al[5], Hübner et al[6], Kapelari et al[7]* and with manufacturer's instructions according to their partitions. We use as statistical tool *EP Evaluator™ software, v. 19.0, USA*.

Results: A total of 7807 TSH determinations were included, 4508 (57%) were from female gender. The median between partitions 0 - 1, 2 - 5, 6 - 10, 11 - 14, and 15 - 18 years was 2.52, 2.70, 2.48, 2.07, and 2.01, respectively. The verification with our population was: 1 - concordant in all partitions with *Lim et al[3]* and *Kapelari et al[7]*; 2- discordant between 1 - 5 years in *Hübner et al[6]*, between 4 - 6 years and 12 - 18 years in *Loh et al[4]*; 3- The manufacturer's label and the study by *Kahapola-Arachchige et al[5]* were discordant in all partitions.

Conclusion: TSH influences many aspects of neurocognitive growth and development in children and adolescents. In this context, the definition of RI by the method used is valuable for standardization in this population. In this study, we verified that the RI evidenced in our database were concordant with *Kapelari et al[7]* and *Lim et al[3]* and some reservations *Hübner et al[6]* and *Loh et al[4]*, with potential use of RI in the laboratory routine. The manufacturer's label did not reproduce RI in this study.

References:

1.CALIPER Reference Interval database - Sick Kids. CALIPER Project

2.*Soldin* SJ, Wong EC, Brugnara C, Soldin OP. *Pediatric reference intervals*: AACCPress, 2011.

3.Lim HH. Korean pediatric reference intervals for FT4, TSH, and TPO Ab and the prevalenceof thyroid dysfunction: A population-based study. Clin Biochem. 2017 Dec;50(18):1256 1259.

4.Loh TP, Sethi SK, Metz MP. Paediatric reference interval and biological variation trends of thyrotropin (TSH) and free thyroxine (T4) in an Asian population. J Clin Pathol 2015;68:642 647.

5.Kahapola-Arachchige K. M., Hadlow N, Wardrop R et al. Age-specific TSH reference ranges have minimal impact on the diagnosis of thyroid dysfunction. Clin Endocrinol, 2012 77: 773 779.

6.Hubner U, Englisch C, Werkmann H *et al*. Continuous age-dependent reference ranges for thyroid hormones in neonates, infants, children and adolescents established using the ADVIA Centaur Analyzer. Clin Chem Lab Med 2002;40:1040 7.

7.Kapelari K, Kirchlechner C, Högler W et al. Pediatric reference intervals for thyroid hormone levels from birth to adulthood: a retrospective study. BMC Endocr Disord. 2008 8:15.

A-160

Reducing Unnecessary Macroprolactin Screening

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

Background: Macroprolactinemia (MP) describes aggregates of prolactin and antibodies which are immunologically detectable but not biologically active. Pretreatment of serum with polyethylene glycol (PEG) to precipitate macroprolactin before testing can avoid this problem. We presently screen all elevated samples for macroprolactin.

This study examined whether the screening process can be improved to avoid unnecessary MP screening.

Methods: The laboratory measures prolactin using 2 Beckman Coulter DxI-800 immunoassay analysers. Locally derived upper 95% reference limits of 274 for men and 478 mIU/L for women are in use. All samples with results above the sex-stratified upper reference limit undergo PEG precipitation (25% PEG 8000 in phosphate buffered saline, 1:1 dilution, 10 minute incubation at room temperature, centrifugation at 2400g for 15 min). Results are compared to locally-derived post-PEG reference intervals (male: 88-318, female: 86-528 mIU/L).

Results: In 3 years, 6729 samples for prolactin measurement were received, of which 2531 were elevated. These all underwent screening for MP, of which 280 were positive. The maximum prolactin concentration encountered was 1166 (female +), 70301 (female -), 1175 (male +), 43330 (male -) mIU/L. If samples with prolactin > 2000 mIU/L were excluded from screening, there would be a reduction of 431 samples.

Conclusion: Macroprolactin screening positivity is unnecessary in samples with extremely elevated Beckman Coulter DxI prolactin concentrations. Using a cutoff < 2000 mIU/L to identify samples for MP screening can reduce screening by 17% while continuing to identify all existing screening positive cases.

A-161

Evaluation of Serum Galectin-3 as a Biomarker of Prediabetes and Complications of Type 2 Diabetes Mellitus

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

Background: Galectin-3 (Gal3) is a β-galactoside-binding protein with reported contradictory protective and damaging biological functions. Currently used as a marker of cardiac fibrosis and heart failure, Gal3 is also known to be associated with Type 2 diabetes Mellitus (T2D) and associated complications. This study explores the clinical utility of Gal3 estimation in subjects with T2D and their apparently healthy first degree relatives (FDR).

Methods: Fasting Gal3, lipid profile, glucose (FPG) and high-sensitivity C-reactive protein (hs-CRP) were measured in 217 (64M, 153F) T2D patients and 226 (106M, 120F). Anthropometric and clinical data were recorded and subjects were classified on the basis of the degree of adiposity and homeostasis model assessment of insulin resistance (HOMA-IR). FDR were classified as normal, prediabetes or diabetic using HbA1c criteria of the American Diabetes Association and T2D were classified by presence or absence of retinopathy, neuropathy (autonomic (AN) and sensory (SN)) and as normo- (NAO, ratio <30mg/g) or; micro-albuminuria (MIA, ratio 30-300mg/g) using urine microalbumin to creatinine ratio. Univariate and multivariate analyses were used to compare study subjects and binary logistic regression with determination of the Odds Ratio (OR)) analyses were used to evaluate associations with T2D complications. Receiver-Operating Characteristic Curve (ROC) analysis was used to evaluate diagnostic utility of Gal3.

Results: Gal3 showed significant ($p < 0.05$) correlations with waist circumference (r=0.21), HbA1c (r = 0.33); glucose (r=0.21) and hs-CRP (r=0.2). In FDR, mean Gal3 levels increased stepwise with increasing glucose intolerance - normal glucose tolerance (7.6ng/ml); prediabetes (8.1ng/ml) and diabetes (9.1 ng/ml) and subjects with HOMA-IR > 2 had significantly higher mean Gal 3 than subjects with HOMA-IR < 2 (10.2 versus 9.3 ng/ml). Binary logistic regression analysis in T2D showed that Gal3 was significantly associated with hypertension (HT) (Odds Ratio (OR) = 1.2); MIA (OR = 1.14); AN (OR = 1.12); SN (OR = 1.2); retinopathy (OR = 1.11). In FDR, ROC analysis showed that Gal3 cut-off value of 8.7 ng/ml has 77% sensitivity and 67% specificity for detection of diabetes. Gal3 significantly ($p < 0.05$) detects HT (Area under the ROC curve (AUC) = 0.709); MIA (AUC = 0.756); AS (AUC = 0.680); SN (AUC = 0.689). Gal3 greater than 9.7 ng/ml was associated with presence of complications with sensitivity and specificity greater than 70%. None of the subjects in this study had clinical evidence of coronary heart disease.

Conclusion: Gal3 is significantly associated with diabetes in FDR and is a strong predictor of associated complications in subjects with T2D. Subjects with Gal3 greater than 9.7ng/ml should be identified as requiring more aggressive management.

A-162

Bucking the Trend - Habitual Coffee Consumption is Associated with Risk Factors for Cardiovascular Disease and Incident Type 2 Diabetes in the Kuwaiti Population

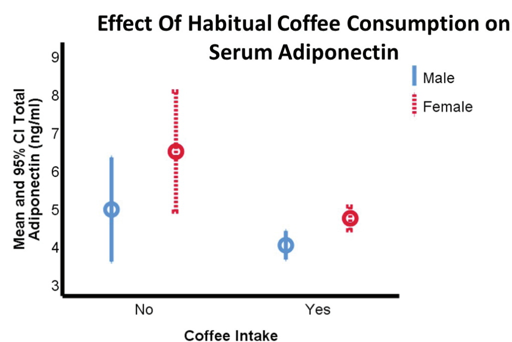
O. A. Mojiminiyi, N. A. Abdella. *Faculty of Medicine, Kuwait University, Kuwait, Kuwait*

Background: Studies in many populations show inverse association of coffee consumption with Type 2 diabetes (T2D) risk. In Kuwait, there is widespread coffee consumption but paradoxically high prevalence of T2D. This study evaluates the associations between coffee intake, incident T2D and metabolic variables in subjects with T2D and their first degree relatives (FDR).

Methods: Fasting glucose (FG), insulin, homeostasis model assessment of insulin resistance (HOMA-IR), lipid profile, HbA1c, adiponectin and high-sensitivity C-reactive protein(hs-CRP) were measured in 607 T2D patients and 622 FDR. Clinical data were recorded. Subjects were classified using coffee intake with number of cups/day, (No Coffee (NC); Coffee Drinker (CD); FDR were classified as normal, prediabetes or diabetic using HbA1c. T2D were classified by diabetes control using HbA1c. Univariate and multivariate analyses were used to compare study subjects and binary logistic regression with determination of the Odds Ratio (OR) was used to evaluate associations with T2D.

Results: In FDR, CD was associated with incident T2D with OR of 1.5, 95% confidence interval 1.2 to 1.7. Mean (SD) HbA1c in T2D was significantly higher in CD than NC (74mmol/mol vs 66 mmol/mol). FPG, Triglycerides, hs-CRP, HOMA-IR,, systolic and diastolic blood pressures were significantly higher in CD than NC whereas HDL-Cholesterol, insulin sensitivity (%S) and adiponectin were significantly lower in CD than NC. In FDR, HbA1c increased with the number of coffee cups per day in a dose-response manner.

Conclusions: The protective associations of coffee described in other populations were not found in Kuwaiti subjects in whom coffee intake is associated with higher risk of T2D and adverse cardiovascular disease metabolic profile. Studies are required to determine the causes of these anomalous findings as variations in coffee bean types, natural composition and preparation techniques may confound the effects of coffee drinking.



A-163

Performance Evaluation for an Automated Assay for the Measurement of Free Testosterone on Diasorin ETI MAX 3000 Analyzer by ELISA Method

S. H. Sobki, S. Saadeddin, M. Sulaiman, T. Al-Mofwaz. *PSMMC, Riyadh, Saudi Arabia*

Background: Testosterone is a C-19 steroid secreted from the testis and the adrenal cortex in men and from the adrenal cortex and ovaries in women. Testosterone is also produced by peripheral tissues from androstenedione, In women however, about half of the circulating testosterone is derived from this origin. Testosterone measurements are used mainly for clinical evaluation of hypogonadism in males and hyper androgenic states in females. Testosterone circulates in the blood bound to three proteins: sex hormone binding globulin, albumin and cortisol binding globulin. Only about 1-2% of the total testosterone remains free. The free testosterone determinations are recommended to overcome the influences caused by variations in transport proteins on the total testosterone concentration. **Objectives:** The objective of the study is to evaluate the performance of ETI-MAX 3000 using Diagnostic Biochem Canada Inc. (DBC) reagents for both adult male and female patients, for the analysis of free testos-

terone. **Method**: Free Testosterone method validation was performed using Diagnostics Biochem Canada (DBC) ELISA KIT on ETI-MAX 3000 analyzer from Diasorin. Method validation was done according to the laboratory policy which follows CLSI guidelines (EP05-A3, EP09-A3, EP06-A, EP17-A2). Precision study was performed using 40 quality control samples of 2 different concentration for a period of 20 days: Mean, SD and %CV were calculated and compared to the manufacturer recommendation. Sensitivity test was performed using 10 samples of zero free testosterone standard, Mean and +2SD was calculated and compared to analytical sensitivity claimed by manufacturer. Method comparison study was done comparing 20 samples with Reference Laboratory. Slope, intercept and correlation coefficient was calculated to check the acceptability of the method. Linearity study was done using 7 different concentration standards (calibrators) samples spanning the analytical measurement range (AMR) from 0.0-60.0pg/ml. Reference range: 20 normal males and 20 normal females samples were analyzed to verify the manufacturer recommended reference range. Acceptable criteria is 90% (18 samples must be acceptable out of 20 sample). Age range 21-48 years old for male and range 19-50 years for female. **Results:** Between days precision study for low and high QC % CV was 8.8 and 5.5 % respectively. Both %CV were consistent with those claimed by manufacturer. The limit of quantitation was observed at less than 0.17pg/ml which agree with the manufacturer claim. Method comparison acceptable criteria slope 0.90-1.10, intercept close to zero and r equal to 0.97. Data was plotted on scatter plot the yield slope was 1.1, intercept 0.251 and correlation coefficient (r) equal to 0.998, all results were within acceptable criteria. Linearity: the method was found linear over the AMR of 0.0-60.0pg/ml. Reference range study: 100% of males and females samples results were within the manufacturer's claim for reference range. **Conclusion:** Overall performance of free testosterone on Diasorin ETI-MAX 3000 was acceptable. It provides reliable results for both adult male and female patients. An estimate of the free testosterone concentration may influence the treatment of hirsutism in women who have a normal testosterone level. As the levels of free testosterone is positively correlated with the degree of hirsutism and virilization in women.

A-164

Determination of Postoperative Hypocalcaemia in Patients Undergoing Total Thyroidectomy by Using a Single Measurement of Pre Closure Plasma Intact Parathyroid Hormone Level

V. A. T. V. Vithana Pathirana. *USJP and PGIM Sri Lanka, Kottawa South, Pannipitiya, Sri Lanka*

Background: Hypocalcaemia is a common complication after total thyroidectomy and produces potentially severe symptoms associated with anxiety and prolongation of hospital stay. So the patient's quality of life will be affected negatively because of the need for long term medication, clinic follow ups and significant cost on treatments.

Methods: Hospital and laboratory based prospective, analytical cross sectional study was conducted including 63 patients who underwent total thyroidectomy, recruited from a surgical unit of a Base Hospital by using a random sampling method. Plasma intact parathyroid hormone level (iPTH), eGFR, serum albumin corrected total calcium (SACTC) and magnesium were analyzed, post operatively. The patients who had pre operative biochemical hypocalcaemia, (SACTC < 2.15mmol/L) hypomagnesaemia, (level < 0.7mmol/L), hypoparathyroidism (level < 14.5pg/mL) and who had eGFR value of less than 60mL/min/1.73m2 were excluded. Plasma iPTH level was measured intra operatively just before the surgical closure and SACTC was measured on post-operative (Postop) D1, and the symptoms of hypocalcaemia were assessed on Postop D1. Data were analyzed using Mini Tab version 16® and descriptive and inferential statistics were used.

Results: The mean age was 48.25 years (SD=12.36) and 89% (n=56) were females and 11% (n=7) were males. 85% (n=55) had benign conditions and 13% (n=8) had malignancies.There was a statistically significant correlation between the intra operative iPTH level and the SACTC on the post operative day 1 with a Pearson correlation value of 0.972 and an adjusted R^2 value of 94.33% ($P < 0.001$). All patients who had intra operative plasma iPTH level of less than or equal to 5pg/ml, got symptomatic hypocalcaemia on Postop day 1. (all patients who had the SACTC level of less than or equal to 1.88mmol/L, developed hypocalcaemic symptoms). All patients who had intra operative plasma iPTH level of more than or equal to 25pg/ml, were normocalcaemic (SACTC >2.15mmol/L) on postop day 1. The regression equation for biochemical hypocalcaemia was, SACTC on Postop day 1= 1.7935 + 0.01582 intra operative plasma iPTH. The sensitivity of detecting Postop symptomatic hypocalcaemia by doing intra operative plasma iPTH was 100% but the specificity was 64.10%. The positive and negative predictive values of this test were 45.28% and 100% respectively. On the other hand the sensitivity of detecting the post operative biochemical hypocalcaemia by doing intra operative plasma iPTH was 71.70% and the specificity was 100%. The positive predictive value and negative predictive values were 100% and 40% respectively.

Conclusion: A significant number of patients developed post operative biochemical as well as symptomatic hypocalcaemia and by performing an intra operative plasma iPTH level, both biochemical and symptomatic hypocalcaemia can be predicted effectively and can start the calcium replacement therapy early. Even though most of the patients developed the biochemical hypocalcaemia post operatively, majority of them did not develop the symptoms of hypocalcaemia. Therefore usually it is not essential to start the calcium replacement therapy on patients with only the biochemical hypocalcaemia and they can be monitored and even can be discharged from the hospital early.

A-165

Evaluation of Thyroid Assay Lot-to-Lot Variability on the Abbott Alinity i Platform

K. Johnson, D. Overcash, T. Roth, K. Wallace. *Abbott Laboratories, Abbott Park, IL*

Background: Thyroid function tests play a critical role in the assessment of thyroid status and the diagnosis of thyroid disease. Many patients with thyroid disease face lifelong treatment and monitoring. It is therefore imperative that these assays show consistent performance over time and multiple reagent lots to impart confidence that fluctuations in values are due to changes in the patients' thyroid status and not to reagent lot-to-lot variability.

Objective: The goal of this study was to evaluate the lot-to-lot performance of five thyroid function assays (TSH, TT4, FT4, TT3, and FT3) on the Abbott Alinity i platform.

Methods: Quality controls (QC) with values across the measurement range and human serum panels targeted at or near medical decision points were tested on each new reagent lot manufactured over a 18- to 23-month period. Assays were run on the Abbott Alinity i instrument. For all assays, QC and panels were tested in replicates of 16 per run. Numbers of runs for each assay varied as follows: TSH, three instruments, one run per instrument; TT4 and TT3, two instruments, two runs per instrument; FT4 one instrument, two runs per instrument; and FT3, two instruments, three runs per instrument. Multiple calibrator lots and, in some cases, QC and panel lots were used over the duration of analysis. Imprecision (percent coefficient of variation (%CV)) was calculated across all reagent lots on each control and panel level mean for each assay.

Results:

Assay	Reagent Lots (n)	Units of Measure	Control Mean Concentration (CV)			Serum Panel Mean Concentration (CV)		
			Low	Middle	High			
TSH	20	uIU/mL	0.10 (6.12%)	6.10 (2.68%)	30.24 (2.22%)	Panel H2 Lot 1: 0.49 (3.03%) Lot 2: 0.48 (4.18%)	Panel N Lot 1: 1.18 (4.64%) Lot 2: 2.38 (2.85%) Lot 3: 2.42 (3.36%)	Panel B 17.74 (2.71%)
TT4	13	ug/dL	4.18 (1.82%)	7.46 (2.11%)	15.35 (3.76%)	Panel N 7.49 (0.95%)		
FT4	16	ng/dL	0.67 (1.90%)	1.23 (2.59%)	2.84 (2.50%)	Panel N 1.16 (0.82%)		
TT3	11	ng/mL	0.73 (2.28%)	1.45 (1.21%)	3.86 (2.13%)	Panel Low 0.68 (1.48%)	Panel Medium 3.06 (1.63%)	
FT3	17	pg/mL	3.10 (2.29%)	6.16 (2.96%)	10.64 (3.01%)	Panel Low 2.11 (2.30%)	Panel High 4.92 (1.60%)	

Conclusions: Each of the five thyroid assays evaluated showed consistent lot-to-lot performance on all controls and human serum panels near important medical decision points. Reliable laboratory results give physicians confidence that changes in thyroid function tests are reflective of a change in patient status and will lead to more informed treatment decisions.

A-166

Long Term Effect of Hormone Therapy on Lab Values in Transgender Individuals

J. A. SoRelle, R. Jiao, E. Gao, P. Day, P. Pagels, N. Gimpel. *University of Texas Southwestern Medical Center, Dallas, TX*

Background: Hormone therapy in transgender individuals induces physical changes, but also changes physiology as reflected by laboratory values. Our previous study demonstrated hormone therapy altered several commonly tested laboratory values at a static point in time. However, we wanted to further examine the durability of these changes in lab values. Defining the point in time when values achieve stability would aid clinician interpretation and allow for establishing transgender specific reference intervals.

Methods: Retrospective chart review of patients attending transgender clinics at local transgender clinics focused on lab values from comprehensive metabolic panel, complete blood count, lipids, and hormone levels. Values were recorded for each visit and grouped by 3-month intervals for the first year and at 6-month intervals up to 5 years. Percent change from baseline was calculated for each lab value. Statistically significant differences were determined by one-way ANOVA with Tukey's post-hoc test (parametric data) or Kruskal-Wallis with Dunn's post-hoc test (non-parametric data) with a p-value <0.05 considered statistically significant.

Results: Baseline and follow up data was available for 88 transgender women and 59 transgender men.

Red blood cells: Hemoglobin, hematocrit and red blood cell (RBC) number significantly decreased within 3 months for transgender women (p<0.0001, baseline vs. 3 months and after) and increased after 6 months for transgender men (p<0.0001, baseline vs. 6 months and after). RBC levels were stable throughout the 5 year follow up period.

Creatinine: Creatinine increased in transgender men after 6 months of testosterone therapy and remained stable through 5 years (p<0.01-0.0001, baseline vs. 6 months and after). Creatinine in transgender women was significantly decreased only at certain timepoints (p<0.05, baseline vs. 9, 12, 18, 30, 42, 54 and 60 months), but clinically remained near baseline levels overall.

Lipids: HDL and LDL were unchanged for up to 5 years in transgender women. However, in transgender men, HDL decreased at early timepoints (p<0.005, baseline vs. 3, 9, and 18 months). Although LDL showed no significant difference in our previous study, it appears to increase during year 5 (p<0.01, baseline vs. 54 and 60 months).

Platelets: Compared to baseline, increased platelets in transgender women was first noticed at 12 months (p<0.05) and continued to increase at 18-30 months (p<0.005) and further at 36 to 60 months (p<0.0001).

Alkaline Phosphatase: We found that alkaline phosphatase decreased in transgender women most significantly at 3-18 months (p<0.0005, baseline vs. 3, 9, 12, 18 months), stayed decreased over the next 2 years (p<0.05, baseline vs. 24-42 months) then returned to baseline after year 4 (p>0.05, baseline vs. 48-60 months).

Conclusion: We present the longest duration of lab values monitored in transgender individuals along with dynamic changes during the 5 year follow up period. During transgender hormone therapy, analytes with the largest shifts (RBCs and creatinine) reach a stable level within 6 months and are stable long term. However, LDL and platelets increase after several years of hormone therapy in transgender women. Some analytes like alkaline phosphatase may increase initially, but return to baseline levels in the long term.

A-167

Effect of Vitamin D Levels in South Indian Population

M. Surla. *Akshaya Hospital, Visakhapatnam, India*

Introduction: Vitamin D plays pivotal role in calcium homeostasis & bone mineral metabolism and has also been implicated in a wide range of biological functions like cell proliferation and differentiation, immune system. Deficiency increases the risk of osteoporosis and other health problems like diabetes, hypertension, cardiovascular disease (heart failure, cardiac death) and certain types of cancer, etc.

Materials and Method: In the study(1) healthy construction labourers, age 25-60 yrs, (n=30, M:F=19:11)were compared with health check-up visiting CARE Hospital, Vishakhapatnam (n:130 M:F=72:88), Individuals were divided into 03 sub-groups - Gr-I: 20-35yrs (n=20, M:F=10:10), Gr-II: 36-50 yrs (n=44, M:F= 17:27) & Gr-III>50yrs (n=66, M:F=26:40)In another study (2) (n=40 M: F=20:20) Gr-I: 25-30 yrs (M: F=10:10) & Gr-II: 55-65yrs (M: F=10:10), Levels of Vitamin D was assayed in individuals before and after supplementation, Deficiency of Vitamin D was orally supplemented for 8 weeks (60,000 IU/week). Serum Vitamin D was assayed by Competitive Electro chemiluminescence immunoassay in Roche e411.

Results: In study (1) Vitamin D levels (ng/ml) in labourers: Mean: 29.70±6.95. Health check up subjects Mean: Gr-I: 14.58±4.99, Gr-II: 15.875.90 & Gr-III: 15.71±6.023 (Health check up vs. labourers, p<0.0001; but no significant difference between different age of the individuals). In study (2) in relation to supplementation Gr-I: 8.96 &31.59±2.90; Gr-II: 5.12±1.27 &31.78±1.34 (before vs. after supplementation, p<0.0001). After 08 weeks Vitamin D supplement was discontinued, Gr-I 7.05±1.00 & Gr-II=9.0±3.00 (no significance between initial vs. after discontinuation)

Conclusion: This study infers that serum Vitamin D levels are not dependent on age and sex. The subjects have one and a half levels, compared to that of the labourers group and hence, dietary source is inferior to continued good sunlight exposure. After 08 weeks of Vitamin D oral supplementation levels reported for health subjects were normal; but after 08 weeks discontinuation, the levels returned back to pre-supplement levels, thus, exposure is necessary and not one time situation of monitoring supplementation.

A-168

Measurement of Hemoglobin A1c by Sebia Capillary Electrophoresis in a Patient with Compound Heterozygosity for Hemoglobins S and G-Philadelphia

Y. G. Nikolaus, D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

Background: Measurement of hemoglobin A1c at our institution is conducted using capillary electrophoresis (CE, Sebia Capillarys2). We received a sample having an uncommon CE pattern with four major hemoglobin peaks, suggesting that the patient was a compound heterozygote for an alpha-chain variant and a beta-chain variant. Because of the unusual nature of this case, we report on our investigation of the sample and its A1c measurement outcome. **Methods:** Measurement of A1c by capillary electrophoresis (CE) for normal subjects involves automated identification of peaks for A1c, A0, and A2. In normal patients that are homozygous for hemoglobin A, there are four electrophoretic peaks: A1c, other A, A0 (the major peak), and A2. Identification of the presence of A2 and other A peaks is a verification condition for use of the electrophoretic pattern for A1c determination. A1c is calculated automatically from the ratio A1c/(A1c+A0) according to a stored calibration curve. In this patient's case, there were four major peaks in addition to A1c, other A, and A2 peaks, suggesting the presence of both alpha-chain and beta-chain hemoglobin variants. The sample was analyzed for hemoglobin variants using HPLC (Trinity Biotech Ultra2, ion exchange column). The sample was also analyzed for A1c by HPLC via calibrated analysis of total glycated hemoglobin (Trinity Biotech Ultra2, boronate affinity method). **Results:** The automated analysis of the sample by CE identified the A1c, A0, and A2 peaks, and returned an A1c measurement of 10.3%. By CE, there were four major hemoglobin peaks present. Two were consistent in position with that commonly observed for hemoglobins A and hemoglobin S, with the remaining two peaks unidentified. Hemoglobin variant analysis by HPLC showed a pattern consistent with the presence of hemoglobins A, S (a beta-chain variant), and G-Philadelphia (an alpha-chain variant), producing four peaks: A (alpha/beta), G (alpha(G)/beta), S (alpha/beta(S)), and hybrid (alpha(G)/beta(S)). Analysis of A1c by the boronate affinity HPLC analyzer returned an A1c measurement of 9.9%. The measurement of A1c by CE and by HPLC were in close agreement, each being compatible with an A1c range of 9.8%-10.4% when allowing for a 5% measurement error (as in CAP accuracy-based proficiency testing standards for A1c). Elevated A1c was consistent with the patient's known history of Type 2 diabetes. The patient had no history of recent blood transfusion. **Conclusions:** Hemoglobin variant analysis for this patient was consistent with compound heterozygosity for hemoglobin S (a beta-chain variant) and G-Philadelphia (an alpha-chain variant). This combination is estimated to be rare (1:125,000), and is clinically benign. The combination leads to four major peaks by HPLC variant analysis and for CE. In this case, automated CE analysis using the Sebia Capillarys 2 instrument was able to correctly identify the minor A1c and the major A0 peaks, leading to a correct A1c% measurement, without interference from the hemoglobin variant peaks. It is important to note that a blood transfusion involving hemoglobin variants must also be considered in cases in which there is more than one major hemoglobin peak seen in CE.

A-169

Development and Evaluation of New Enzymatic HbA1c Assay

T. Hayashi, M. Shiota, T. Fujita, M. Horio, H. Soya, N. Ogawa, T. Sano, T. Ogawa, M. Shiida, Y. Katayama. *Kyowa Medex Co. , Ltd. (July 1,2019: The company's name was changed to Hitachi Chemical Diagnostics Systems Co. , Ltd.), Shizuoka, Japan*

Background: Hemoglobin A1c (HbA1c) is a species of glycated hemoglobin which is used as a biomarker for diabetes mellitus. At present, HbA1c is measured by several methods, such as HPLC, LATEX immunoturbidimetric assay, and enzymatic assay. The HbA1c enzymatic assay has an advantage that there is no pollution of a reaction cuvette in an autoanalyzer and a calibration process is very simple. The current HbA1c enzymatic assay comprises the following steps: i) degrading HbA1c with a protease in the presence of a denaturing agent to generate a fructosyl dipeptide fragment, fructosyl valyl histidine (F-VH), ii) oxidizing the liberated F-VH with fructosyl peptide oxidase (FPOX) to generate glucosone, valyl-histidine and hydrogen peroxide (H_2O_2), iii) reacting the generated H_2O_2 with a chromogen in the presence of peroxidase (POD) to produce a dye. We have created a new oxidase named HbA1c direct oxidase (HbA1cOX) that acts on whole HbA1c molecule as its substrate from FPOX by combined structure-based site specific mutagenesis and random mutagenesis (Ogawa et al. *Sci. Rep*. **9**, Article number: 942, 2019). In this study, using HbA1cOX, we developed a new enzymatic HbA1c assay on an autoanalyser without the necessity of protease and evaluated the performances of the assay.

Method: A kit for enzymatic HbA1c assay consisting of the first reagent and the second reagent was prepared: the first reagent containing a denaturing agent of HbA1c and a chromogen, and the second reagent containing HbA1cOX and POD. Accuracy was evaluated using commercially available domestic samples for quality assurance with NGSP-assigned reference value (5-12%) on JCA-BM9130 (JEOL). Linearity and repeatability were evaluated by using materials from human blood cells on JCA-BM9130. Comparison between the new enzymatic assay on JCA-BM9130 and HPLC analysis (G8, Tosoh) was carried out by using whole blood samples. For the purpose to investigate whether reaction time in this assay can be set shorter than that in conventional enzymatic HbA1c assay, performances of this assay was carried out on 7170S (Hitachi High-Technologies) under the conditions of both 10 minutes (conventional reaction time) and a shorter period, 6.5 minutes.

Results: The new HbA1c enzymatic assay showed a good accuracy using samples with NGSP-assigned reference values and met the certification of NGSP (ratio to reference value <±6.0%). Good linearity level for this assay was observed in the range of 4.0% to 17.0% HbA1c. Repeatability was ranged from 0.2% to 1.0% of CV. This assay had a good correlation with HPLC analysis (r=0.99). Moreover this assay showed good performances even under the condition of shorter reaction time.

Conclusion: The new enzymatic HbA1c assay using HbA1cOX showed a good performances. This assay which does not require protease has some advantages that: (i) there is no carry over and contamination of protease to following reagents via an operating autoanalyser; and (ii) reaction time can be set shorter than conventional enzymatic HbA1c assay. Research and development for practical applications of the new enzymatic HbA1c assay using HbA1cOX are in progress.

A-170

Comparison of Hemoglobin Variants across Siemens Healthineers HbA1c Assays

A. Tyler, C. Schaible, C. Robinson, J. Gisiora, J. Jones. *Siemens Healthineers, Newark, DE*

Background: In 2015, according to the World Health Organization, 1.6 million deaths occurred that were directly related to diabetes, and in 2014, an estimated 422 million adults were living with diabetes globally. Along with a healthy diet and regular exercise, early diagnosis is important in delaying the onset of type 2 diabetes. Glycated hemoglobin (HbA1c) is a form of measurement of glycemic states that reflects the average blood glucose level over the preceding 8–12 weeks. HbA1c is formed by a nonenzymatic Maillard reaction between glucose and the N-terminal valine of the β-chain of HbA, whereby a labile Schiff base is formed and converted into the more-stable ketoamine (irreversible) via an Amadori rearrangement. Siemens Healthineers offers HbA1c assays on the following laboratory diagnostic systems: the Atellica® CH Analyzer*, ADVIA® Chemistry Systems, Dimension® Integrated Chemistry Systems, and Dimension Vista® Intelligent Lab Systems*.

Method: A hemoglobin variant study was performed on the following Siemens Healthineers assays: Atellica CH A1c_E, ADVIA Chemistry A1c_E, Dimension A1C, and Dimension Vista A1C assays. A minimum of 20 samples for each of the following variants were tested according to CLSI protocol EP07-A2: HbA2, HbC, HbD, HbE, and HbS. Samples were obtained from the National Glycohemoglobin Standardization Program (NGSP) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC).

Results: The hemoglobin variants study yielded the following overall mean biases across all variants:

Atellica CH A1c_E Assay: –4.61 to –0.14% bias

ADVIA Chemistry A1c_E Assay: –1.05 to –2.88% bias

Dimension A1C Assay: –2.27 to –0.17% bias

Dimension Vista A1C Assay: –1.08 to 0.79% bias

Conclusion: Hemoglobin A1c assays offered by Siemens Healthineers demonstrated little to no significant interference across all systems and variants tested. Overall mean bias was <5% across all systems and variant types.

*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

A-171

Low Carbohydrate and High Fat or Keto Diet Effect on HbA1c Levels in Type2 Diabetics

K. L. Angati. *CARE Hospitals, Visakhapatnam, India*

Introduction: Low carbohydrate high Fat(LCHF)_or Keto diet was originally developed almost 100 years ago to treat epilepsy. Nowadays, it is used as a nutrition plan by health-conscious men and women to optimize body composition and athletic performance. Recent research suggests that high fat, very-low carb diets have another benefit help control glucose, triglycerides, insulin, and body weight in people with diabetes.

Aim: The aim of the study is to know the impact of Keto diet on Hb1C of patients with Type 2 diabetics. The study had participants with type2 diabetes restrict their carbohydrate intake to 20g or less daily, increase fat, and maintain a modest but not high protein intake to induce a state referred to as "nutritional ketosis".

Material and Method: 130 type2 DM patient with HbA1c level 7.8%±1.3, mean glucose level 169md/dl ±31 are made to follow LCHF diet for 3months of duration HbA1c level are estimated on ortho clinical Diagnostics Vitro's 5.1Fs analyser Enzymatic method, Result: After 3months of follow up 15 dropouts 5 no effect remaining 110 show decrease in HbA1c level to 6.43%±1.47 & mean glucose level 137mg/dl±28, p value <0.0010 shows significant variation, linear regression for HbA1c -r2: 0.53, glucose r2: 0.49.

Conclusion: No adverse effects were observed except for mild constipation. The number of patients on drugs decreased from 3 at baseline to 1 or nil at 3months. No patient required inpatient care or insulin therapy. In summary, the 5-10%-carbohydrate diet over 3 months led to a remarkable reduction in HbA1c levels. ketogenic diet leads to greater improvements in symptoms associated with type II diabetes.

A-172

Comparison of Two Vitamin D Immunoassays to Detect 25-OH Vitamin D2 and D3

E. Garnett[1], J. Li[2], D. Rajapakshe[1], E. Tam[1], Q. H. Meng[2], S. Devaraj[1]. *[1]Baylor College of Medicine, Houston, TX, [2]UT MD Anderson Cancer Center, Houston, TX*

Background: Assessment of Vitamin D status by measurement of 25-Hydroxyvitamin D (25-OH-D) is widely performed by immunoassay. Yet, the ability of these assays to detect Vitamin D2 (as 25-OH-D2) or Vitamin D3 (as 25-OH-D3) varies. It is important to recognize the ability of an assay to quantitate either form of 25-OH-D to evaluate Vitamin D status, as patients with Vitamin D deficiency may be supplemented with Vitamin D2 or D3. We compared recovery of 25-OH-D2 and 25-OH-D3 by two assays in our medical center, and determined bias of these assays against LC-MS/MS measurements.

Methods: The Abbott Architect i1000 SR 25-OH Vitamin D assay and Roche Cobas 8000 Vitamin D assay were used for this study. Pooled serum (n ≥ 7) from samples previously tested at Texas Children's Hospital for 25-OH-D was spiked with 50 ng 25-OH-D2, 25-OH-D3, or solvent control, and recovery of either 25-OH-D species was calculated. Reference samples from ARUP laboratories (n = 20) with known endogenous concentrations of either 25-OH-D2 or D3 as determined by LC-MS/MS

were also measured for total 25-OH-D to calculate bias between our assays and LC-MS/MS.

Results: Recovery of 25-OH-D3 in spiked samples was similar by Architect (78-82%) and Cobas (61-78%). Recovery of 25-OH-D2 was lower than 25-OH-D3, and was poorer by Architect (35-38%) than by Cobas (56-66%). In comparison to manufacturer-specified cross-reactivity, both assays performed more poorly than indicated, particularly in measurement of 25-OH-D2. Median Architect cross-reactivity with 25-OH-D2 was 36% compared to a claim of 82%, while Cobas cross-reactivity was 64% compared to a claim of 92%. In measurement of samples with known 25-OH-D concentrations, performance of Architect and Cobas assays was similar for 25-OH-D3, with median bias of 18.4% for Architect and 14.0% for Cobas. In measurement of samples with known 25-OH-D2, median bias was -21.0% for Architect and 19.0% for Cobas. For samples with concentrations > 20 ng/mL 25-OH-D2, the Architect assay exhibited large negative bias (-40.9%).

Conclusion: Correct classification of Vitamin D status relies on accurate detection of both 25-OH-D2 and 25-OH-D3 by the assay in use. While the Architect and Cobas assays performed similarly in detection of 25-OH-D3, both assays performed poorly in detecting 25-OH-D2, Architect performing more poorly than Cobas, with poorer recovery and significant negative bias at higher concentrations of 25-OH-D2. This is a point of concern for appropriate monitoring of Vitamin D2-supplemented patients, as 40% of reference samples with known sufficient Vitamin D (30-60 ng/mL), were classified as Vitamin D insufficient (21-29 ng/mL) by Architect, while the Cobas assay correctly classified these samples. These findings agree with previous studies, and indicate that caution should be used in interpreting Total 25-OH-D results in patients supplemented with Vitamin D2. There is a need for manufacturers to improve their Vitamin D immunoassays to match the gold standard LC-MS/MS tests.

A-173

Precision and Comparability of Three Fertility Assays on Seven Immunochemistry Systems

P. Findeisen[1], I. Zahn[1], M. Barral Juez[2], D. Krempel[1], M. T. Haro Romero[2], T. De Haro-Muñoz[2], C. Garcia Rabaneda[2], K. Klopprogge[3], S. Engelmann[3]. *[1]Laboratory Dr. Limbach and Colleagues, Heidelberg, Germany, [2]UGC Análisis Clínicos intercentros, Hospital Universitario Campus de la Salud, Granada, Spain, [3]Roche Diagnostics GmbH, Mannheim, Germany*

Background: In our study we evaluated precision and comparability of three fertility markers (Estradiol, Progesterone, Testosterone) on seven different immunochemistry analysers at two sites in Europe. The included analysers were: **cobas e** 801 system (Roche Diagnostics GmbH), **cobas e** 601 system (Roche Diagnostics GmbH), ARCHITECT i2000SR (Abbott), UniCel DxI 800 (Beckman Coulter), Liaison® XL (DiaSorin), ADVIA Centaur XPT and IMMULITE 2000 XPi (Siemens Healthineers).

Methods: The testing protocol covered CLSI precison (5-day scheme) and a technical method comparison. For determination of the intralaboratory precision, pooled quality control material from Bio-Rad at three different concentration levels per analyte were distributed to both sites. Testing was done on five days in 5-fold determinations per assay using the pooled sample material. CVs were calculated per site as repeatability and within-lab precision. For method comparisons, aliquots of pooled native sample material covering a broad concentration range per analyte were distributed to both testing sites and measured on the respective instruments.

Results: For the precision, tested with Bio-Rad control materials, the following intermediate precision CV ranges were calculated: Estradiol (~336 pmol/L - 1420 pmol/L), 1.2% CV on **cobas e** 801 system to 13.2% CV on ADVIA Centaur XPT; Progesterone (~ 2.2 nmol/L - 78 nmol/L), 1.9% CV on **cobas e** 801 system to 15.9% CV on UniCel DxI 800; Testosterone (~2.2 ng/mL -11.5 ng/mL), 1.6 %CV on **cobas e** 801 system to 9.2% CV on ADVIA Centaur XPT. Very good result comparability was demonstrated between the cobas systems using the same method: Passing/Bablok regression: slope 0.97 - 1.01, Pearson's r correlation: 0.997 - 0.999. The comparability of the **cobas e** 801 system to other tested methods was also investigated: Passing/Bablok regression slopes ranging from 0.72 (IMMULITE 2000 XPi) to 1.42 (UniCel DxI 800), Pearson's r correlation 0.984 (UniCel DxI 800) to 0.998 (Liaison® XL).

Conclusion: The data of our study support laboratories in assessing the precision and comparability of their routine fertility methods.

A-174

Reference Intervals for Thyroid-stimulating Hormone, Free Thyroxine, and Free Triiodothyronine in Elderly Chinese Persons

D. Wang, Y. yu, c. Ma, h. li, l. qiu, x. cheng, x. guo, y. yin, d. li, z. wang, y. hu, s. lu, g. yang, h. liu. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

Background: Thyroid hormone levels are essential for diagnosing and monitoring thyroid diseases. However, their reference intervals (RIs) in elderly Chinese individuals remain unclear. We aimed to identify factors affecting thyroid-stimulating hormone (TSH), free triiodothyronine (FT3), and free thyroxine (FT4) levels using clinical "big data" to establish hormone level RIs for elderly Chinese individuals.

Methods: We examined 6781, 6772, and 6524 subjects aged ≥65 years who underwent FT3, FT4, and TSH tests, respectively, at Peking Union Medical College Hospital between September 1, 2013 and August 31, 2016. Hormones were measured using an automated immunoassay analyzer (ADVIA Centaur XP). RIs were established using the Clinical Laboratory Standards Institute document C28-A3 guidelines.

Results: The median TSH was significantly higher in women than in men; the opposite was true for median FT3 and FT4 levels. No differences were observed in TSH or FT4 by age in either sex or overall; FT3 levels significantly decreased with age. Seasonal differences were observed in TSH and FT3 levels but not FT4 levels; the median TSH was the highest in winter and lowest in summer, whereas the median FT3 was the lowest in summer (albeit not significantly). RIs for TSH were 0.53-5.24 and 0.335-5.73 mIU/L for men and women, respectively; those for FT3 were 3.76-5.71, 3.60-5.42, and 3.36-5.27 pmol/L in 64-74-, 75-84-, and 85-96-year-old subjects, respectively. The RI for FT4 was 11.70-20.28 pmol/L.

Conclusion: RIs for TSH in elderly individuals were sex-specific, while those for FT3 were age-specific.

A-175

Correlation between Oxidative Stress, DNA Damage & Cancer Risk in Type 2 Diabetes: A Case Controlled Study in Riyadh, KSA

A. O. ALJOHI[1], M. ABUDAWOOD[2], S. Sobki[1]. *[1]Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [2]KING SAUD UNIVERSITY, Riyadh, Saudi Arabia*

Background: Type 2 Diabetes Mellitus (T2DM) also known as non-insulin dependent diabetes is the most common endocrine disorder worldwide characterized by hyperglycemia, insulin resistance and deficiency. It is a heterogenous metabolic disorder. Abnormal lipid peroxidation and altered lipid homeostasis are more common in diabetes and are aggravated with a poor glycemic control. Chronic hyperglycemia promotes oxidative stress which represents a major pathophysiological link in progression of T2DM. Oxidative stress results either due to production of oxygen radicals or due to enhanced lipid peroxidation exceeding the scavenging capacity of antioxidant enzymes. Furthermore, oxidative stress can result in DNA damage leading to high risk of cancer. **Methods**: The aim of the present study was to evaluate status of oxidative stress, DNA damage and cancer biomarkers in regard to hyperglycemic state in T2DM patients and to correlate the glycemic state with cancer. A total of 150 subjects consisting of control (50) and T2DM patients (100) were enrolled. Additionally, three tertiles were created among the two groups based on levels of HbA1c (Tertile I=5.37±0.34, n=50; Tertile II=6.74±0.20, n=50; Tertile III=9.21±1.47, n=50). Oxidative stress parameters including malondialedehyde (MDA) and antioxidant enzymes-superoxide dismutase, catalase, glutathione peroxidase were measured. Damage to DNA was analyzed by measuring the levels of DNA damage adduct-8 hydroxy deoxy Guanosine (8-OHdG). To detect cancer resulting from oxidative stress, cancer biomarkers CEA, AFP, CA125, CA-15, CA19-9 and prolactin were measured in these subjects. All measurements were analysed by SPSS software. **Results:** Levels of MDA and antioxidant enzymes altered significantly in T2DM subjects compared to the control at $p<0.001$ and $p<0.05$ level of significance. Significant DNA damage accompanied with elevated levels of CEA, CA19-9 and decreased CA125, AFP and prolactin were noted in T2DM group. Serum levels of CA 19-9, and CEA increased at $p<0.05$, whereas levels of prolactin decreased significantly ($p<0.001$) in T2DM compared to control. Additionally the mean values of DNA damage adduct 8-OHdG differ significantly at $P<0.01$ between the two groups. However, no significant correlation in oxidative stress parameter, antioxidant enzymes, DNA damage and neither with the highest tertile of HbA1c (>7.5%) was noted. **Conclusion:** Based on the results obtained in the present study, we conclude that there is considerable change in oxidative stress and DNA damage in T2DM patients compared to healthy control. Hence, assumption that the oxidative stress could cause cancer in T2DM as a result of hyperglycemic state was not speculated in this study.

A-177

Metabolic and Biochemical Parameters in Patients with Skin Tags

S. Rana, B. k. yadav, D. P. Shrestha. *Institute of Medicine, Teaching Hospital, Kathmandu, kathmandu, Nepal*

Background: Acrochordon or fibroepithelial polyp, commonly known as Skin tags (STs) are one of the most common benign skin condition, consisting of skin projecting from the surrounding skin, usually occurring on the eyelids, neck and axillae, less often on the trunk and groin. Skin rubbing, skin aging and a familial predisposition are causes for STs, while others described hormonal imbalances and hyper-insulinemia as contributing factors. Studies have found an association of STs with conditions such as obesity, diabetes mellitus and atherogenic lipid profile. Abdominal obesity and the consequent insulin resistance are said be important contributing factors for diabetes, dyslipidemia and cardiovascular disease.

Objective: To highlight the association of metabolic parameters (body mass index, blood pressure, waist circumference) and the biochemical parameters (lipid profile, fasting glucose, HbA1c and serum leptin) levels in Nepalese patients with STs visiting the Dermatology out patient department of Teaching Hospital, Kathmandu, Nepal.

Methods: This study comprised of 99(men or women) presenting to the dermatology clinic where 15 males and 35 females with STs taken as cases and 14 males and 35 females of the same age and sex with no STs were taken as controls. Metabolic parameters (body mass index, blood pressure, waist circumference) along with the Biochemical parameters (serum lipid profile, glucose, HbA1c, and serum leptin) were measured in all individuals. SPSS ver. 20.0 was used to analyze the data. Mann-Whitney U test was applied for comparison of median to see the difference between case and control group and Spearman's correlation was used to establish the association between two quantitative variables.

Results: Serum leptin was found to be significantly higher in both male and female patients having STs than the controls at the probability level of 0.001. Also, serum leptin is seen to increase with increasing BMI in both male and female cases and controls.

In male with STs fasting blood glucose, glycosylated hemoglobin, triglyceride, systolic blood pressure and diastolic blood pressure was found higher than the individuals without STs. In female fasting blood glucose, glycosylated hemoglobin, total cholesterol, triglyceride, systolic blood pressure and diastolic blood pressure was found higher than the individuals without STs.

Conclusion: In the present study, there is significant association of STs with triglycerides, total cholesterol, blood pressure and serum Leptin levels. It is thus implied that skin tags may be one of the important skin markers of metabolic disorders and may attract physicians and dermatologist for further investigation as it is proved to be not just a cosmetic problem.

This leads us to recommend the change of life style of patients with STs and or hyperlipidemia, as stopping active smoking and prevention of passive smoking, regular exercises, weight reduction, changing carbohydrate diets into high protein diets.

A-178

IRS2 rs1865434 Variant is Associated with Adiposity and Insulin Resistance in Brazilian Subjects

C. M. Fajardo[1], R. H. Bortolin[1], A. Cerda[2], R. C. C. Freitas[1], R. de Oliveira[1], T. I. Moraes[1], V. B. Malaquias[1], M. H. Hirata[1], R. D. C. Hirata[1]. *[1]University of São Paulo, São Paulo, Brazil, [2]University of La Frontera, Temuco, Chile*

Background: Obesity has worldwide distribution and causes health, social and economic problems. It causes metabolic alterations and inflammatory state, which are closely related to hypertension, diabetes, dyslipidemia and cardiovascular disease. Polymorphisms in genes involved in leptin and insulin signaling, which regulate glucose homeostasis, body energy and adiposity, have been targets of studies on obesity and diabetes. However, the molecular mechanisms are not well known. Thus, the aim this study was to analyze the influence of the variants in *IRS2* (rs1865434, C>T) and *MC3R* (rs3746619, C>A) on adiposity, inflammatory and adipokine profiles in the Brazilian population. **Methods:** Two-hundred-forty-one subjects, age 28 to 68 years, 23.0% man and 77.0% women, were recruited. Clinical and anthropometric data were recorded, and peripheral blood was obtained for laboratory and genetic analyses. Biochemistry variables, such as glucose, lipids, fibrinogen and hsCRP, were determined using laboratory automation systems. HbA1c was determined by high performance liquid chromatography (HPLC), insulin was determined by a chemiluminescence method and soluble leptin receptor (sLEPR) were determinate by ELISA. Inflammatory markers (PAI-I, IL-6, IL-1β, TNFα) and adipokines (leptin, adiponectin and resistin) were analyzed by multiplex solid phase immunoassays. American Diabetes Association criteria were used to classify subjects for insulin resistance (IR) and obesity. *IRS2* (rs1865434, C>T) and *MC3R* (rs3746619, C>A) polymorphisms were analyzed by real-time PCR. PolymiRTS Database was used to predict the functional effect of the *IRS2* 3'UTR variant (rs1865434) on interaction with regulatory miRNAs. **Results:** Carriers of the *IRS2* C allele (CC+CT genotype) had higher values of body mass index (BMI), waist circumference (WC), basal metabolic rate (BMR), insulin and HOMA-IR and lower concentration of sLEPR than whose carrying T allele (TT genotype) ($p<0.05$). The *IRS2* C allele was more frequent in IR subjects (72.3%) than in non-IR subjects (27.7%, p=0.014). A stepwise logistic regression analysis showed that C allele (OR:2.01; CI:1.02-2.99; p=0.044), dyslipidemia (OR:9.14; CI:4.49-18.60; $p<0.001$) and hypertension (OR:2.97; CI:1.39-6.33; p=0.005) increased the risk for IR. *MC3R* C>A polymorphism was not associated with obesity or IR in this population. The *IRS2* rs1865434 3'UTR variant disrupts the binding site for hsa-miR-3145-3p and hsa-miR-3191-5p and creates a new site of binding for hsa-miR-6817-3p. **Conclusion:** *IRS2* rs1865434 variant is associated with adiposity and IR and could be a helpful biomarker to evaluated early diagnosis of metabolic status in Brazilian subjects. The association of *IRS2* rs1865434 with metabolic alterations could be explained by a new profile of interactions with miRNAs at the 3´UTR.

A-180

Measurement of GlycoMark® 1,5-anhydrogulcitol from Dried Blood Spot Specimens

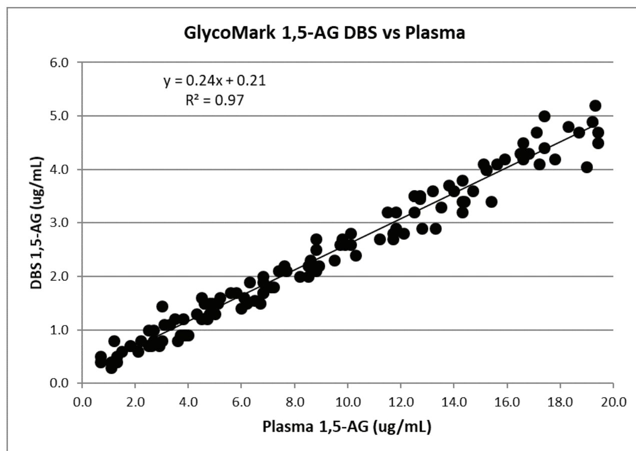
J. Dahlen[1], S. Kazmierczak[2]. *[1]Hikari Dx, Inc., San Diego, CA, [2]DiabetOmics, Hillsboro, OR*

Background: 1,5-anhydroglucitol (1,5-AG) is a glycemic control marker uniquely specific for identifying hyperglycemic excursions and glycemic variability occurring in the prior 1-2 weeks. Blood samples for 1,5-AG measurements are typically collected at the same time as other labs. Currently, there is no commercially available in-office or point-of-care test for measuring 1,5-AG, and careful management of poor glycemic control requires additional blood draws. Testing from dried blood spot (DBS) samples may be an effective way to facilitate sample collection and transport to a laboratory by patients or healthcare providers, without the need for skilled phlebotomists. The present study evaluates the viability of using DBS specimens with the GlycoMark 1,5-AG assay.

Methods: Whole blood samples from 120 donors with plasma 1,5-AG concentrations up to 20 μg/mL were spotted onto Whatman 903 filter paper and dried overnight at room temperature. Eight 3.1 mm punches were made from each DBS sample and eluted for 2 hours at room temperature in phosphate buffered saline (PBS) containing polyacrylic acid. All measurements were performed using the GlycoMark test on a Beckman AU480 analyzer. A comparison of 1,5-AG measurements from the eluted DBS samples and matched plasma samples was performed using linear regression. Functional sensitivity, linearity, and sample stability were also evaluated.

Results: Comparison of the DBS 1,5-AG and plasma 1,5-AG yielded a slope of 0.24 and correlation coefficient of r^2 = 0.97. The linearity study yielded a slope of 0.24 and correlation coefficient of r^2 = 0.99. The functional sensitivity was 1 μg/mL. DBS samples were stable for 6 days at room temperature, which was the maximum duration tested.

Conclusion: DBS samples appear to be a viable sample type that provides added convenience for testing with the GlycoMark 1,5-AG assay, demonstrating a high correlation with plasma measurements, at least 6 day sample stability, and acceptable linearity and functional sensitivity.



A-181

Performance Evaluation of Roche Cobas c513 HbA1c Analyzer

M. Lim, S. Lim, S. P. Hoo, C. Gan, T. C. Aw. *Changi General Hospital, Singapore, Singapore*

Background: The Roche cobas c513, a standalone high throughput HbA1c analyzer that is capable of producing up to 400 tests/hr. It is solely dedicated to HbA1c testing and aids situations where there is increased demand for quick HbA1c results. Our laboratory currently performs whole blood HbA1c testing on the Cobas c502 module, which is part of the cobas c8000 autoanalyzer (Roche Diagnostics). HbA1c testing is often subject to congestion as the EDTA sample is shared with Hematology for blood cell counting. Besides, the c8000 accepts multiple sample types and performs a myriad of other chemistry tests. In addition, the introduction of a new closed tube sampling feature on the cobas c513 analyzer minimizes user intervention from sample registration to result delivery. We evaluated the c513 and describe our experience. **Method:** The Roche HbA1c assay for both c513 and c502 are immunoassays based on immuno-turbidimetry. The performance of HbA1c on c513 was verified – precision (coefficient of variation – CV%), linearity, limit of blank (LOB), limit of detection (LOD) and correlation studies and compared against the c502. A throughput study with 50 pre-programmed HbA1c samples was carried out. Statistical analyses were performed using EP evaluator v11. **Result:** The inter-assay CV for HbA1c on the c513 was 1.2% at HbA1c level of 5.7% and 0.8% at HbA1c level of 10.5%. The analytical measuring range was linear and verified for HbA1c concentrations from 4.5%-18.2%. Correlation and regression analyses showed very close agreement between c513 and c502 (r = 0.9973, slope = 1.009). The Cobas c513 took 17 minutes to complete all 50 HbA1c analyses as compared to 40 minutes on the cobas c502. **Conclusion:** The cobas c513 is a good system for high volume HbA1c testing and is by far the fastest with the added feature of closed tube sampling. As a standalone analyzer, the delivery of HbA1c results on the c513 will not be impacted by any technical issues on other modules as currently faced with the c8000. Having a better balance of tests on different platforms and isolating EDTA whole blood sampling from serum/plasma testing on the c8000 has been salutary for our laboratory operations.

A-182

Subclinical Thyroid Dysfunction, Bone Mineral Density, and Osteoporosis in a Middle-Aged Korean Population

K. Lee, S. Lim, H. Park, H. Woo, M. Kwon. *Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Seoul, Korea, Republic of*

Background: Thyroid dysfunction is associated with loss of bone density, osteoporosis, and a high risk of fracture. The connection with subclinical thyroid dysfunction, however, is still controversial. The present study examined the relationship between subclinical thyroid dysfunction and bone mineral density (BMD) in middle-aged healthy adults.

Methods: A total of 25,510 healthy Koreans with normal free thyroxine levels were enrolled from January 2011 to December 2016. The average age of the 15,761 women was 45, and the average age of the 9,749 men was 48. Levels of thyroid-stimulating hormone and BMD measurements were recorded in all subjects. BMD was measured using dual-energy X-ray absorptiometry. Subclinical hyperthyroidism was defined as a TSH level < 0.27 µIU/mL and subclinical hypothyroidism was defined as TSH > 4.2 µIU/mL. Stepwise multiple regression analysis was performed to find the determinants for lumbar spine BMD. Multiple logistic regression analysis was performed with osteoporosis (T-score ≤ -2.5) or osteopenia (T-score from -2.4 to -1.0) as a dependent variable.

Results: Mean serum levels of TSH and fT4 were 2.3 ± 1.6 µIU/mL and 1.2 ± 0.1 ng/dL for women, and 2.0 ± 1.5 µIU/mL and 1.3 ± 0.2 ng/dL for men, respectively, with no significant differences between female and male subjects. The frequencies of subclinical hyperthyroidism and hypothyroidism were 0.6% and 8.4% for women, 0.5% and 5.7% for men, respectively. No apparent association was found between subclinical thyroid dysfunction and BMD at the lumbar spine, femur-neck, and proximal femur sites compared with a euthyroid group. Age, body mass index (BMI), and postmenopausal status affected BMD in women and only BMI affected BMD in men. Subclinical hypothyroidism was independently associated with a lower risk of osteoporosis (odds ratio 0.657, 95% confidence interval 0.464-0.930) in 4,710 postmenopausal women. From men age 50 and older, lower BMD (T-score ≤ -2.5 or T-score from -2.4 to -1.0) groups were not significantly associated with subclinical thyroid status. Among those who visited our institute more than twice during study periods, 2,082 subjects (1,445 women and 637 men) showed persistent euthyroid status and 88 subjects (54 women and 34 men) showed persistent subclinical hypothyroid status. There was no difference in mean lumbar spine BMD between the two groups in euthyroid versus subclinical hypothyroid: 1.140 g/cm2 vs 1.119 g/cm2 in women, 1.185 g/cm2 vs 1.183 g/cm2 in men, respectively. The persistent subclinical hypothyroidism group had a higher rate of BMD reduction than the persistent euthyroid group, although statistically not significant (-0.09% vs -0.23% in %BMD change per year).

Conclusion: No apparent association was found between subclinical hypothyroidism or subclinical hyperthyroidism and BMD at the lumbar spine and femur in a large cohort of middle-age men and women. Subclinical hypothyroidism was independently associated with a lower risk of osteoporosis in postmenopausal women.

A-183

Stability Validation and Reference Intervals for a Mass Spectrometric Assay of Random Urinary Fractionated Metanephrines for Screening of Pheochromocytoma and Paraganglioma

K. Wang, X. Gao, H. Cong, X. Shi, W. Zhang, Z. Zhou. *Fuwai Hospital*, *Peking Union Medical College*, *Chinese Academy of Medical sciences, Beijing, China*

Background: The tumor of pheochromocytoma and paraganglioma(PPGL)can secrete excessive catecholamine, which can lead to secondary hypertension and serious complications of heart, brain and kidney. Accurate etiological diagnosis is helpful for targeted treatment and improvement of prognosis, the endocrine society clinical practice guideline of PPGL recommend 24-hours(24-h) urinary fractionated metanephrines as one of the first choices for laboratory qualitative diagnosis, but it's not convenient for a patient to collect the 24-h sample,to solve this problem, we developed the clinical reference interval of random urinary fractionated metanephrines adjusted by creatine, and compared its diagnostic value with 24-h urinary metanephrines.

Methods: Random urine and 24-h urine were collected from patients with primary hypertension (n=145) and patients suspected for PPGL (n=44). Deuterated metanephrine and normetanephrine as internal standard were added to samples before solid phase extraction (SPE)and LC-MS/MS analysis. We studied the sample storage stability,developed the clinical reference intervals of random urinary fractionated metanephrines adjusted by creatine, and the diagnostic performance was compared with the 24-h urinary fractionated metanephrines.

Results: Urinary metanephrines are stable at room temperature and at 4°C for at least 4 days, based on this stability experiment, we think the results of 24-h urinary fractionated metanephrines are accurate without adding any preservative. We suggest (8.24ug/g-240.96ug/g)and(40.23ug/g-634.32ug/g)as the clinical reference intervals of random urinary fractionated metanephrine and normetanephrine adjusted by creatine, The sensitivity and specificity of the combined diagnosis were 95.4% and 99.2% respectively(AUC=0.998), compared with clinical diagnosis, the kappa index was 0.954(P<0.05). Compared between 24-h urinary fractionated metanephrines and random urinary fractionated metanephrines adjusted by creatine showed good correlation(r=0.961 for metanephrine and r=0.966 for normetanephrine).

Conclusions: Random urinary metanephrines are stable, patients can collect samples without adding any preservative. Random urine can also provide accurate diagnostic information, and random sample collection greatly facilitates the patients, shorten diagnostic time and may save medical expenses indirectly.

A-184

Performance Evaluation of the VITROS® TSH3* Assay on the VITROS® 5600/XT 7600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems

C. Thomas[1], R. Stevens[1], L. West[1], E. Oliver[2], J. Pang[1], H. Griffiths[1]. [1]*Ortho-Clinical Diagnostics, Bridgend, United Kingdom,* [2]*Ortho-Clinical Diagnostics, Rochester, NY*

Background: In-Vitro Diagnostic (IVD) manufacturers and the International Federation of Clinical Chemistry Committee for the Standardisation of Thyroid Function Tests (IFCC C-STFT) have been participating in a collaboration to harmonise thyroid stimulating hormone (TSH) assay results across assay platforms. We are developing a new harmonised, fully automated assay for the measurement of TSH in human serum and plasma on VITROS® Systems.

Methods: The VITROS® TSH3 assay uses a one-step immunometric technique where TSH present in the sample reacts simultaneously with a streptavidin conjugated antibody bound by Biotin-BSA on the wells, and an antibody-horseradish peroxidase conjugate. The antigen-antibody complex is captured by the antibody coated on the wells. Unbound materials are removed by washing, 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 electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is directly proportional to the concentration of TSH in the sample. The time to first result in the system is 24 minutes.

Results: Preliminary data indicates an assay range of 0.0060-150 µIU/ml. The Limit of Blank, Limit of Detection and Limit of Quantitation (20% CV) of 0.0030, 0.0058 and 0.0063 µIU/ml respectively were established according to CLSI EP17-A2. Correlation between VITROS® TSH3 and the All Procedure Trimmed Mean (APTM) values of the IFCC follow-up patient sample panel was obtained with the 81 samples within the measuring range. The regression statistics, using Passing & Bablock, were as follows VITROS® TSH3 = 0.98 APTM TSH Value (µIU/ml) -0.0078; Pearson Correlation Coefficient (r) = 1.00. In a CLSI-EP05 A3 precision study, testing of six precision pools (n=40) produced total imprecision ranging from 4.2 to 15.5 %CV.

Conclusion: In summary, the VITROS® TSH3 assay is harmonised to the IFCC follow-up panel APTM values, and shows acceptable performance on the VITROS® 5600/XT 7600 Integrated and VITROS® 3600 and ECi/ECiQ Immunodiagnostic Systems. *In development.

A-185

Influence of Polymorphisms *LEP* c.-2548A>G and *LEPR* c.668A>G on Metabolic Alterations in a Pediatric Population of Southern Chile

R. Dominguez Crespo Hirata[1], R. Brito[2], V. Manríquez[2], N. Aguilar-Farias[2], D. Chandía-Poblete[2], J. Navarro[2], C. Moreno[1], M. Hiroyuki Hirata[1], A. Cerda[2]. [1]*University of Sao Paulo, Sao Paulo, Brazil,* [2]*Universidad de la Frontera, Temuco, Chile*

Background: Chile is the country with the highest prevalence of child obesity in Latin-America. Genetic biomarkers are useful to early risk evaluation of obesity and its relationship with metabolic alterations, mainly genetic variants in the leptin-melanocortin pathway which is involved in the regulation of satiety, energy and glucose metabolism. Polymorphism in genes that encode leptin (*LEP*) and leptin receptor (*LEPR*) has been associated with obesity and metabolic syndrome (MetS) in different populations. We evaluated the influence of *LEP* c.-2548A>G and *LEPR* c.668A>G on the risk of obesity, MetS and anthropometric and biochemical parameters in Southern Chilean pediatric population.

Methods: Two-hundred and eight individuals aged 9-13 years were grouped as normoweight (BMI z-score -1 to +1, n=60), overweight (BMI z-score +1 to +2, n=79) and obese (BMI z-score >+3, n=69) or according to MetS status using the Cook's modified criteria. Clinical and demographic data, anthropometric measures and pubertal development according Tanner were registered by a pediatrician. Biochemical parameters such as lipid profile, glycaemia, and insulin were determined by commercial available laboratory methods. HOMA-IR was also calculated. *LEP* c.-2548A>G and *LEPR* c.668A>G genotypes were determined by real-time PCR using allelic discrimination assays.

Results: The prevalence of overweight (38%) and obesity (33.2%) was high; however no association was observed between *LEP* and *LEPR* variants and obesity risk or anthropometric measures in this population. MetS (12.2%) was only present in obese individuals and a higher frequency of the allele G for the *LEPR* c.668A>G polymorphism was observed in this group (MetS: 87.5.%, non-MetS: 1.2%; p=0.020). A multiple logistic regression analysis demonstrated increased risk of MetS for individuals carrying the G allele (AG and GG genotypes) compared to those with the *LEPR* c.668AA genotype (OR: 6.65; 95%CI: 1.05 - 42.30; p=0.027). *LEPR* c.668 G allele carriers had higher concentration of triglycerides (TG) (*LEPR* c.668G carriers: 107±76 mg/dL, AA: 80±36 mg/dL; p=0.016), which was confirmed by a multiple lineal regression analysis using relevant covariates (β=24.1 mg/dL, SE=10.2 mg/dL; p=0.019). A multiple logistic regression analysis introducing high TG (>75th percentile) as a dependent variable using clinical covariates showed that *LEPR* c.668 G allele was associated with increased risk of high TG (OR: 15.5; 95%CI: 1.97 - 122.30; p<0.001). No association with biochemical variables was observed for the *LEP* c.-2548A>G variant.

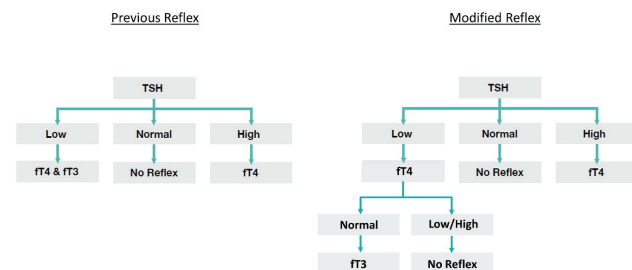
Conclusion: Although no association with obesity was observed the *LEPR* c.668G allele was associated with increased risk of MetS and hypertriglyceridemia in the study population. The *LEPR* c.668A>G polymorphism may be useful as an early biomarker of cardiometabolic risk in pediatric Chilean population. Nevertheless, the results reported here should be explored further in studies using larger sample sizes to confirm these observations.

A-186

Choosing fT3 and fT4 Wisely: A Data Driven Reflexive Testing Approach to Reduce Thyroid Hormone Testing

J. Taher[1], J. Gilmour[2], L. K. Hicks[2], D. Brinc[3], D. Beriault[2]. [1]*University of Toronto, Toronto, ON, Canada,* [2]*St. Michael's Hospital, Toronto, ON, Canada,* [3]*University Health Network, Toronto, ON, Canada*

Background: Choosing Wisely and the American Thyroid Association recommend a thyroid stimulating hormone (TSH)-centered approach for the assessment of thyroid hormone status. Based on this recommendation, a laboratory-based reflexive testing algorithm was implemented at St. Michael's hospital. The algorithm reflexed for free thyroxine (fT4) and free triiodothyronine (fT3) with low TSH, and fT4 with high TSH. Minimal reduction in fT3 was attributed to unnecessary reflexed fT3 that were added-on. The aim of the current quality improvement initiative was to further decrease inappropriate free thyroid hormone testing by optimizing the TSH reflex algorithm and test ordering requisitions. **Methods:** To optimize sensitivity and specificity, the use of TSH reference intervals (0.4-5.5mIU/L) as reflex cut-points was assessed by receiver operating characteristic (ROC) curves. To reduce unnecessary fT3 tests, the current reflex was adapted to reflex fT3 only when TSH was low and fT4 was normal. To reduce unnecessary reflexive testing, a 'TSH only' option was implemented into test ordering requisitions. **Results:** Optimal low and high TSH cut-points of 0.4mIU/L and 5.7mIU/L were identified by ROC curve analysis respectively, thereby confirming the appropriateness of using the TSH reference range to initiate the reflex. FT4 testing decreased by 16% (230 vs 192/month) 4 months post-implementation, whereas an unexpected increase in reflexed fT3 by 30% was observed (n=117 vs 153/month) due to an error in the 2nd tier of the reflex. Implementation of the 'TSH only' option reduced reflexive tests by 23% (n=2151 vs 1665/month) with only 2 add-on calls to the laboratory as a balancing measure. **Conclusions:** Reflex testing algorithms may require hospital-specific modifications to achieve maximal reduction in unnecessary free thyroid hormone testing. This can be further supported by appropriate reflex cut-points and the option to order reflex-independent TSH. A pre-implementation test environment and post-implementation result monitoring is important to identify unintended outcomes.

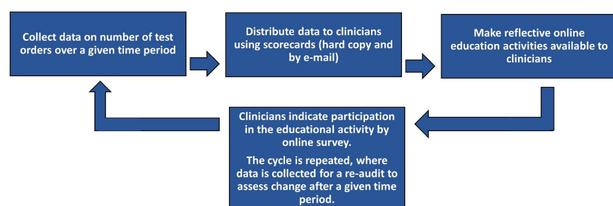


A-187

Best Practices in Medicine (BPiM): An Audit and Feedback Approach to ‘Right Size’ Laboratory Test Utilization

J. Taher[1], M. Pasic[2], D. Arab-Obrien[2], C. Barnes[2], M. Jackman[1], A. Kapur[2], R. Kherani[3], J. Maniate[4], S. Mulsant[2], R. Seth[2], E. Wooster[2], V. Tron[2]. *[1]University of Toronto, Toronto, ON, Canada, [2]Unity Health Toronto, Toronto, ON, Canada, [3]University of British Columbia, Richmond, BC, Canada, [4]The Ottawa Hospital, Ottawa, ON, Canada*

Objectives: Test ordering continues to increase disproportionately to demographic shifts. While increased test ordering has become a primary target, both underutilization and overutilization can contribute to serious healthcare outcomes. The BPiM project focuses on exploring utilization rates of laboratory testing and diagnostic imaging. The primary aim of the project is to ‘right size’ resource utilization by increasing or decreasing as appropriate. **Methods:** Retrospective data collection, data analysis and scorecard output was completed using a Python computer programming code for thyroid stimulating hormone (TSH) orders. Departmental and individual clinician information collected from a 3 month period included: total test orders, total abnormal results, percent abnormal results and number of repeat tests within 90 days. This information was included in a personalized scorecard that was subsequently distributed to clinicians across 13 departments. An online learning activity containing practice guideline information and a self-reflective survey were also made available. One month later, test ordering data was prospectively collected over the same time period, and new scorecards were distributed. Scorecards for 25-hydroxyvitamin D, rheumatoid factor, and anti-cyclic citrullinated peptide were also delivered with data analysis to be carried out in early 2019. **Results:** Online educational activities and surveys had a 30% response rate. Despite a non-significant change in total test orders (n=2707 vs 2440; p=0.07), there was an overall increase in percent abnormal test results (17% vs 37%; p<0.001), supporting a change towards appropriate test ordering practices. Furthermore, a decrease in 90 day repeat test results was observed with the internal medicine practitioners (average n=3.4 vs 1.5; p=0.01). **Conclusions:** The BPiM approach can improve clinician performance by targeting appropriate resource utilization using a continuing professional development framework. Ultimately, this framework will be used to develop and implement an automated internal auditing system.



A-188

Performance Evaluation of the ADVIA Centaur Androstenedione Assay

L. Parker[1], J. Driscoll[1], C. Higgins[1], M. Stranz[1], K. Garvey[1], P. Donovan[2], Z. Zhao[1], Y. Zheng[1]. *[1]Siemens Healthineers, Newark, DE, [2]Siemens Healthineers, Walpole, MA*

Background: Androstenedione is a 19-carbon steroid that serves as a precursor for testosterone and estrone. It is primarily synthesized from dehydroepiandrosterone (DHEA) via 3ß-hydroxysteroid dehydrogenase in the ovaries, testes, and adrenal glands.

Androstenedione is most commonly used in conjunction with other steroid assays to evaluate the function of the adrenal glands and ovaries or testes and to determine the cause of symptoms of androgen excess. It is also used in the monitoring of treatment for congenital adrenal hyperplasia.

A new ADVIA Centaur® Androstenedione (ANDRO) assay for the measurement of androstenedione in human serum and plasma has been evaluated by Siemens Healthineers. The studies below describe performance of the assay on the ADVIA Centaur XP Immunoassay System.

Methods: The ADVIA Centaur ANDRO assay is a fully-automated competitive immunoassay using direct chemiluminescent technology. Reagents include a sheep monoclonal antibody coupled to paramagnetic particles in the solid phase and a novel acridinium ester in the Lite reagent. Solid phase and Lite reagent are incubated with 20 µL of patient sample. Competition for solid phase binding occurs between androstenedione in the sample and the Lite reagent. Separation follows, and the amount of signal generated is inversely proportional to the concentration of androstenedione in the sample. The time to first result is 18 minutes.

Results: The ADVIA Centaur ANDRO assay correlated well to LC-MS/MS across the measuring interval of 0.30 to 9.00 ng/mL using Passing-Bablok regression. Equivalent performance was determined for serum, lithium heparin plasma, and potassium EDTA plasma sample types. Within-lab precision was <8% CV (with 95% confidence), and the assay demonstrated good specificity, with ≤10% interference and ≤1% cross-reactivity for the majority of the compounds evaluated. Stability data demonstrated a calibration interval and onboard stability of 35 days.

Conclusions: The ADVIA Centaur ANDRO assay demonstrates good precision, specificity, and correlation to LC-MS/MS.

*Information about this device is preliminary. Safety and effectiveness for the uses discussed have not been established. The device is under development and not commercially available. Future availability cannot be ensured.

A-189

Analytical Interference of Hypertriglyceridemia in the Evaluation of Hepatic Injury Markers in Oncological Patients: Case Reporter

V. O. Fernandes[1], M. C. G. CASTELO[2], L. V. Martins[1], A. D. R. MONTENEGRO[1], A. B. C. Bruno[1], L. F. F. Aragão[1], R. M. Montenegro Júnior[1], L. L. A. Cavalcante[2], S. P. Bandeira[2], G. A. Campana[3]. *[1]UFC, FORTALEZA, Brazil, [2]DASA, FORTALEZA, Brazil, [3]DASA, São Paulo, Brazil*

Objective: To describe a clinical case in which it is suggested that the analytical interference of hypertriglyceridemia led to the falsely elevated clinical analysis of gamma-glutamyltransferase (GGT), aspartate aminotranspherase (AST) and alanine aminotranspherase (ALP), interfering with clinical management in oncology patients. Clinical case: Patient, 14 years old, male, diagnosis of Generalized Congenital Lipodystrophy (LCG) and Langerhan Cell Histiocytosis was submitted to chemotherapy and corticoid therapy. After the fifth chemotherapy cycle, presented hypertriglyceridemia and elevation of GGT, AST and ALT (table 1). The cause of hypertriglyceridemia was probably due to severe insulin resistance related to the underlying disease (LCG) and exacerbated by the pulses of steroid therapy. Due to possible hepatotoxicity, the chemotherapy was interrupted. Patient was hospitalized, submitted to the treatment of hypertriglyceridemia and to the hepatic and biliary tract investigation. Viral serology and imaging tests were negative. With the progressive decrease of the triglycerides, a concomitant reduction of GGT and transaminases was observed, suggesting that no hepatic damage occurred and raised possible analytical interference with lipemia. Discussion: Hypertriglideridemia is characterized by turbidity of serum or plasma and is an important interference in laboratory analysis. Several methodologies may suffer from lipemia interferences. The spectrophotometry is probably the most affected because lipoprotein particles in the sample can absorb light. The amount of absorbed light is inversely proportional to the wavelength and decreases from 300 to 700 nm. Methods that use lower wavelengths are more affected. Clinical chemistry methods like ALT and AST use reaction NAD(P)+ ↔ NAD(P)H + H+ as an indicator reaction for determining concentration or activity of the analyte. Since the change of absorbance is measured at 340 nm, most of these methods are strongly affected by lipemia. The present report ratifies the importance of medical knowledge with regard to the preanalytic, analytical and post-analytical interferences of clinical practice.

Table 1

Date	Triglycerides (mg/dL) RI: <90*	GGT (U/L) RI: 15-63*	AST (U/L) RI: ≤34*	ALT (U/L) RI: 10-49*
09/08/2018	7320	2480	NA°	NA
09/15/2018	8290	3242	216	320
09/19/2018	NA	2879	242	222
09/20/2018	NA	NA	250	251
09/24/2018	407	1289	95	110
10/08/2018	323	469	91	107
10/18/2018	519	493	144	176
10/20/2018	185	270	79	94
11/19/2018	199	176	51	85
12/10/2018	233	177	45	63

*Methods: Triglycerides - GPO, trinder, without serum white; GGT, AST and ALT – IFCC modified
RI: Reference Interval °not available

A-190

Reverse Triiodothyronine (rT3) Quantification in Blood Serum for Research Purposes by LC-MS/MS using Liquid-Liquid Extraction following Protein Precipitation

J. M. Di Bussolo[1], X. Xie[1], R. Castillo[2], A. Mustafa[2], H. Othman[2]. *[1]Thermo Fisher Scientific, San Jose, CA, [2]BioReference Laboratories, Elmwood Park, NJ*

Background: Reverse triiodothyronine (rT3) is an inactive isomer of the most potent thyroid hormone triiodothyronine (T3). Both are made from thyroxine (T4). For scientists studying the metabolic consequences of starvation and critical illness, we report an LC-MS/MS research method to quantify rT3 from 5 to 60 ng/dL of blood serum after protein precipitation (PPT) and liquid-liquid extraction (LLE).

Methods: To precipitate proteins and extract analytes, 250 µL aliquots of specimens were vortexed with 250 µL of water, 600 µL of acetonitrile and 200 µL of methanol containing rT3-$^{13}C_6$ internal standard (IS) before mixing 1.2 mL of ethyl acetate. After centrifugation, 2 mL of the organic layer of each were collected and dried. Residues were reconstituted with 150 µL of 25% acetonitrile in water and transferred a microtiter plate. 50 µL of each extract were injected into a heated (60°C) 100 x 2.1 mm column packed with solid-core silica particles with C_{18} bonded phase. A 6-minute water-to-methanol gradient containing 0.1% formic acid separated rT3 and IS from T3 and other interfering compounds and eluted them into the heated ESI source of a MS/MS system. Selected-reaction monitoring of two transitions for rT3 (651.8 > 605.8 for quantitation and 651.8 > 507.9 for conformation) and for IS (657.8 > 611.8 and 657.8 > 513.9) occurred within a 0.8-minute data window. Ion ratios were calculated from peak areas measured by these transitions to help verify peak purity.

Results: The desired measuring range from 5 to 60 pg/mL was consistently linear ($r2 \geq 0.99$ with 1/X weighting). Carryover was less than 0.5%. The lowest limit of quantitation (LLOQ) was 2 ng/dL, significantly below the concentration of the lowest calibrator (4.5 ng/dL). Ion ratios among calibrators and QCs averaged 0.53 for rT3 and 0.39 for IS, and donor specimens calculated to have rT3 no less than the LLOQ had ion ratios within +/- 20% of 0.53. Among 50 donor specimens analyzed, IS peak areas averaged 67% of the average IS peak areas among calibrators and QCs within the same batch, indicative of ion suppression by matrix interferences. However, IS peak ion ratios throughout the batch were well within +/- 20% and the average difference was 2.7%, indicating adequate compensated for ion suppression by the IS. Intra- and inter-batch precisions among 20 replicate injections from three pools (low, medium and high rT3 levels) were less than 6% and 8% CV, respectively. Correlation of rT3 concentrations in 64 donor samples determined by our research method with those from a reference lab were excellent. Values ranged from 6 to 56 ng/dL. Only 1 out of 64 results differed by 21%, all others were less than 20% and averaged -2.1%, which is a small negative bias for our research method.

Conclusion: Robust, reliable and sensitive quantification of rT3 in donor serum samples prepared using PPT and LLE was achieved using a four-channel LC-MS/MS system. A single channel had a throughput of 9 injections per hour, which can be doubled, tripled or quadrupled based on throughput requirements.

A-191

Comparative Study among Values Obtained from Vitamin 25(OH)D for Curitiba’s Population, in the Summer and Winter Seasons in 2018

T. F. C. Pereira, C. C. Albuquerque, L. B. A. Guzzo, A. T. S. Pereira. *Laboratório Frischmann Aisengart, DASA, São José dos Pinhais - PR, Brazil*

Background: Vitamin D is a pre-hormone mainly responsible for calcium homeostasis in the body. The largest source of this compound is obtained by exposing the skin to UVB photons (ultraviolet B, 290-315 nm) from the sun. Several studies have shown that hypovitaminosis D is a public health problem and can reach about 50% of the world population and is associated with several pathologies and metabolic disorders. Curitiba is one of the brazilian cities with lower incidence of solar rays, high cloudiness and less sun during the day. This study analyzed the hypovitaminosis D in the city of Curitiba. **Methods:** Were use 2700 results from first-time individuals who were tested at the Frischmann Aisengart Laboratory in 2018. These samples were classified into summer (January to March) and winter (July to September) collections, and correlated by sex (female and male) and age (A - 0 to 20 years, B - 21 to 40 years, C - 41 to 60 years, D - above 60 years). The values considered for the evaluation of hypovitaminosis D are based on the recommendations of the Brazilian Society of Endocrinology and Metabology (SBEM). The information was organized in Excel 2016 worksheet and statistically analyzed using the non-parametric Chi-square test ($p < 0.05$). **Results:**It was observed that 67.7% of the studied population had deficiency or insufficiency of vitamin 25 (OH) D, with an average value of 26.9 ng/mL. In summer this scenario is 53.4% and in winter it reached 82%. There was a negative correlation when the male and female sexes were compared. However, when classified by age groups, there was a positive correlation ($p < 0.001$), with the lowest age group in both seasons, summer and winter, 28,1 ng/mL and 22.4 ng/mL, respectively, followed by age groups A, C and D. In the summer, the age group B had the highest deficiency and insufficiency index with 61.5%, however, in the winter, the age group A had the highest rate with 88.5%. The age group D had the lowest deficiency index and insufficiency of the study, being 43.5% in summer and 70.2% in winter.

Conclusion: The present study corroborates other studies demonstrating the concern with hypovitaminosis D in urban centers, combine with the importance of evaluating this metabolite at all ages, in addition to diagnosis and efficient treatment to avoid damages caused by vitamin D deficiency.

A-192

Serum 25- Hydroxyvitamin D Status in the Tropical City of Fortaleza/ Ceará/ Brazil

L. L. A. Cavalcante[1], M. C. G. CASTELO[1], T. A. C. Palhano[1], F. L. B. Furtado[2], S. P. Bandeira[1], G. A. Campana[3]. *[1]DASA, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]DASA, São Paulo, Brazil*

Background: Hypovitaminosis D is highly prevalent. Epidemiological data suggest that this prevalence may be lower in areas exposed to greater light. Objective:To determine the 25- hydroxyvitamin D (25(OH)D) serum status in pediatric, adult and elderly population from Fortaleza, Brazil. Methodology: A cross-sectional study was conducted by Diagnostics of America (DASA). The records of 25(OH)D were evaluated in the database of DASA laboratory present in all health districts of Fortaleza from 2015 to 2017. Fortaleza is located in a region of tropical climate with an annual average temperature of 26.5 ° C and latitude of -3.7183°. Vitamin D dosages were performed on the ADVIA Centaur XP platform Siemens. The patients were divided into categories by sex, age groups (less than 2 years, between 2 and 18 years, from 18 years to 60 years and over 60 years) and in serum levels of 25(OH)D. The subjects were characterized as hypovitaminosis following the criteria of the Official Positioning of Brazilian Society of Clinical pathology/ Laboratory Medicine and the Brazilian Society of Endocrinology and Metabolism (2018): < 20 ng/mL (<50 nmol/L) for healthy population (up to 60 years) and < 30 (< 75 nmol/L) for at-risk groups such as elderly (older than 60 years) and infants. Serum levels of vitamin D were described in mean and standard deviation. Results: 293,331 samples were analyzed. There was a predominance of females in all groups evaluated. The mean of serum levels of 25(OH) D in each age group analyzed are described in table 1. Conclusion: Our findings show that the average 25(OH)D in this population is in level of sufficiency. Due to the use of databases, defintive comments cannot be made in the interest of more complete data.

Table 1

Age Groups	< 2 years	2 - 18 years	18 - 60 years	> 60 years
Mean and standard deviation 25(OH)D	36,6 ±10,7ng/mL (90±25nmol/L)	29,9 ±10,6ng/mL (72±25nmol/L)	29,8 ±11,4ng/mL (72±27nmol/L)	31,3 ±10,3ng/mL 72±27nmol/L)

A-193

Comparison of Long Term Variation of Siemens Vista A1c Patient Data to Long Term Patient Variation of Sebia Capillarys 2 Flex Piercing and Roche Tina Quant Gen II A1c

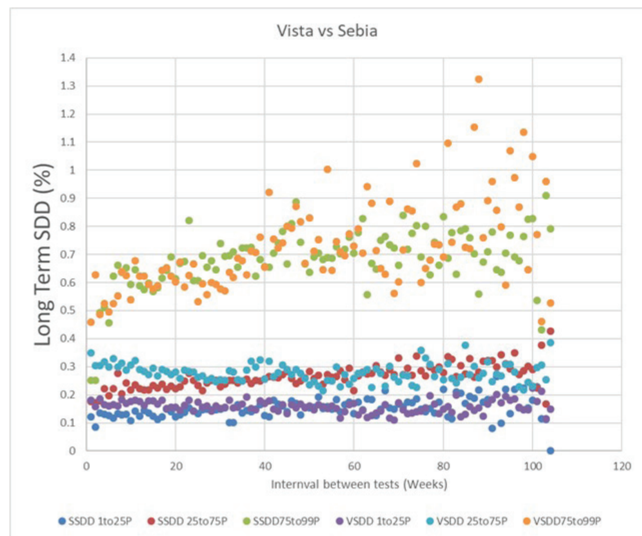
J. Mei[1], G. Cembrowski[2], R. Guerin[3], M. Cervinski[4], C. McCudden[5]. *[1]University of Alberta, Winnipeg, MB, Canada, [2]University of Alberta, CC Quality Control Consulting, Edmonton, AB, Canada, [3]CSSS Chicoutimi, Chicoutimi, QC, Canada, [4]Giesel School of Medicine, Hanover, NH, [5]University of Ottawa, Ottawa, ON, Canada*

Introduction: Recently, we demonstrated that the statistical analysis of sequential intra-patient data can yield realistic measures of long term (LT) patient biologic and analytic variation. To accomplish this, we determined all possible intrapatient pairs reported by the laboratory for at least 2 years and sorted these pairs by time between their sequential assays. The standard deviation of duplicates (SDD) was determined and charted for each time interval. We apply this analysis to the Siemens Vista A1c assay and compare its LT variation to LT variation of two other assays determined in other laboratories.

Methods and Materials: 3.5 years of Siemens Vista de-identified HbA1c (226,000 values from 129,749 Ottawa patients) were analyzed. The LTSDD analysis was performed on 3 subsets of the Ottawa A1c data: low normal (1 to 25 percentile), adequate glycemic control (25 to 75 percentile) and poor control (75 to 99 percentile). The graphs of the LT intra-patient SDD for the 3 patient subpopulations were compared to graphs generated in New Hampshire (Roche Tina Quant Gen II) and Quebec (Sebia Capillarys 2 Flex Piercing).

Results: The Figure shows a composite graph of the Vista and Sebia assays for the three subpopulations. The Vista assay demonstrates higher variation in both the low normal and adequate control populations especially in testing intervals under 6 months. For the poor diabetes control patients, the SDD variations overlap.

Discussion: Compared to the Sebia and Roche assays, Vista exhibits excess analytic variation in patients with nonelevated or good control A1c. Analytical sources of this variation include between instrument and between reagent lot variation. The magnitude of these differences in variation is roughly 0.1 divided by 6.5 or about 1.5%. Future evaluations of the performance of HbA1c assays should include LT estimates of variation derived from stratified patient data.



A-194

Comparison between Two Automated Methods for the Estimation of Vitamin D

G. V. Kapoula[1], I. A. Stergiou[2], I. G. Moisoglou[3], S. T. Provatopoulou[4], P. G. Bagos[1]. *[1]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece, [2]Department of Biochemistry, General hospital of Lamia, Lamia, Greece, [3]Quality assurance and Continuing Education Unit, General Hospital of Lamia Greece, Lamia, Greece, [4]Department of Nephrology, Lamia General Hospital,, Lamia, Greece*

Background: Determination of 25(OH)D, is useful for determining whether a patient is vitamin D deficient, sufficient or intoxicated. It is the only vitamin D metabolite that is used as a reliable indicator of vitamin D status and to monitor these conditions. A comparative study was performed between assay methods in two automated analyzers: a) Liaison Diasorin and b) i2000 Architect Abbott. The aim of the study was to clinical evaluate the two 25(OH)D chemiluminescent immunoassays Methods:80 serum blood samples were collected from patients attending the outpatient department of our hospital. The samples were measured simultaneously in both analyzers, at the same day. The patients were 85% male with an average age of 35 years. Regression and correlation analysis was performed using the statistical package STATA.

Results: results showed a high degree of correlation between the two methods (correlation coefficient r=0.93). Regression analysis yielded a regression coefficient of 25(OH)D Liaison on 25(OH)D i2000 Architect of 0.81(95%CI;0.74-0.89).

Discussion: Correlation analysis showed a strong positive association between the two method assays. However, 25(OH)D assays values cannot be used interchangeably when measured with different assays mainly due to discrepancy in methodologies and different reagent specificity.

A-195

Evaluation of the Thyroid Assays on the Abbott Alinity I Immunoassay Analyzer

C. S. Lau, L. Y. C. Wee, N. B. Ahmad, Y. L. Liang, T. C. Aw. *CHANGI GENERAL HOSPITAL, Singapore, Singapore*

Background: Abbott Diagnostics recently introduced the Alinity I immunoassay analyser. The Alinity is a compact immunoassay system that employs chemiluminescence technology. It is intended to replace the established Abbott Architect i2000SR analyzer. It has a smaller footprint, is more ergonomic and generates test results faster. We evaluated the Alinity's analytical performance for the most commonly requested thyroid tests in our laboratory - thyroid-stimulating hormone (TSH), free thyroxine (fT4), free tri-iodothyronine (fT3), anti-thyroid peroxidase (TPO-Ab) and thyroglobulin antibodies (Tg-Ab).

Methods: The performance evaluation included assay linearity, limit of quantitation (LoQ), analytical precision (3 levels of controls), and correlation with the current Architect TSH, fT4, fT3, TPO-Ab and Tg-Ab assays. Statistical analyses were performed on MedCalc software v18.11.3 (MedCalc, Ostend, Belgium).

Results: The Alinity assays were linear for TSH from 0.013-92.0 mIU/L, fT4 from 6.7- 84.9 pmol/L, fT3 from 1.2-24.2 pmol/L, TPO-Ab from 5.7-992.6 IU/mL and Tg-Ab from 5.4-962.2 IU/mL. The LoQ corresponding to an assay coefficient of variation (CV) of 20% was 0.0025 mIU/L for TSH, 3.0 pmol/L for fT4, 0.2 IU/mL for TPO-Ab and 0.3 IU/mL for Tg-Ab; for fT3 the CV was 9.5% at 1.1 pmol/L. Inter-assay (n=20) precision assessed using 3 pools of control sera (Table 1). Regression analyses (Passing-Bablok, n=50) showed: TSH Alinity = 0.964x+0.0983, fT4 Alinity = 0.958x+0.657, fT3 Alinity = 1.050x-0.403, TPO-Ab Alinity = 1.142x-0.612 and Tg-Ab Alinity = 1.045x+0.026. The correlation coefficients were close - TSH r=0.9998, fT4 r=0.9961, fT3 r=0.9821, TPO-Ab r=0.9979 and Tg-Ab r=0.996. The upgraded software and new functions on the Alinity such as continuous loading or unloading of reagents and supplies without interrupting instrument operations improves laboratory productivity and efficiency.

Conclusion: The performance of TSH, fT4, fT3, TPO-Ab and Tg-Ab assays on the Alinity is good, within the manufacturer's claims, comparable to the Architect assays and fit for operational use.

Table 1. Interassay Precision for Alinity Thyroid Assays

ANALYTE (units)	Level 1 (CV%)	Level 2 (CV%)	Level 3 (CV%)
TSH (mIU/L)	0.1±0.01 (2.0)	6.0±0.6 (1.5)	30.0±2.9 (3.0)
fT4 (pmol/L)	7.6±0.12 (1.6)	21.3±0.36 (1.7)	37.7+1.22 (3.2)
fT3 (pmol/L)	5.9±0.27 (4.5)	8.0±0.32 (4.0)	23.1±2.14 (9.2)
TPO-Ab (IU/mL)	20.5±0.91 (4.4)	47.5±1.62 (3.4)	68.8±2.40 (3.5)
Tg-Ab (IU/mL)	12.4±0.44 (3.5)	49.0±1.29 (2.6)	81.2±2.67 (3.3)

A-196

Development of a New Biochip Based Immunoassay for the Detection of Parathyroid Hormone Applied to the Evidence Evolution Analyser

L. Speers, T. McHugh, H. Winter, S. Lynch, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: Parathyroid Hormone (PTH) is key for the homeostasis of calcium and phosphorous in the body via its direct action on the bones and kidneys. Precise detection and quantification is important for the differential diagnosis of primary hyperparathyroidism, secondary hyperparathyroidism and hypoparathyroidism**.** Secondary hyperparathyroidism is a common complication in Chronic Kidney Disease (CKD) patients, which if not monitored sufficiently can result in Mineral Bone Disorder. With the aim to introduce new analytical approaches to facilitate the monitoring of PTH levels in clinical settings, this study reports the development of a biochip based immunoassay applied to the fully automated random access Evidence Evolution analyser.

Methods: A chemiluminescent sandwich immunoassay, defining a discrete test site on the biochip surface, was employed and applied to the Evidence Evolution analyser. In accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2, Limit of Blank (LOB), and Limit of Quantitation (LOQ), the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %, were used to determine assay sensitivity. Intra-assay precision was determined by running 20 replicates of three levels of precision material across the assay range. Plasma patient samples (*n*=90) were assessed to complete a correlation study against another commercially available immunoassay.

Results: Initial assessment of assay sensitivity established the LOB and LOQ to be 6.5 pg/mL and 11.2 pg/mL. Intra-assay precision was found to be the following for each level of precision material, PM1 6.5 % CV (79.4 pg/mL), PM2 4.8 % CV (377.1 pg/mL), PM3 4.6 % CV (920.0 pg/mL). Linear regression analysis of the data from the correlation study gave an r value of 0.98 for EDTA plasma patient samples (n=90) ranging from 3.3-1315.1 pg/mL.

Conclusion: The results show the applicability of the developed biochip based PTH immunoassay on the fully automated, high throughput, Evidence Evolution analyser for the reliable detection of PTH from EDTA plasma samples. The PTH assay will aid in the management of CKD patients by complementing Randox's already established vitamin D and CKD I assays for the Evidence Evolution platform.

A-197

Thyroid Stimulating Autoantibody Profile after Treatment of Graves' Disease with Radioiodine

C. E. S. Ferreira[1], L. B. Fiorin[2], T. S. Kasamatsu[2], I. S. Kunii[2], G. K. Furuzawa[2], M. L. V. Castiglioni[3], R. M. B. Maciel[2], L. K. Matsumura[2], R. P. Furlanetto[2], M. C. Batista[1], J. R. M. Martins[2]. *[1]Laboratório Clínico, Hospital Israelita Albert Einstein, São Paulo, Brazil, [2]Centro de Doenças Tiroidianas e Laboratório de Endocrinologia Molecular e Translacional, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil, [3]Medicina Nuclear, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil*

Background: Serum levels of anti-TSH receptor antibodies (TRAb) rise after treatment of Graves' disease (GD) with radioiodine (131I). However, it is poorly understood whether part of these autoantibodies are stimulatory (TSI) and what is the impact of their persistence on treatment outcome. In this study, we used a new TRAb assay that detects only stimulating antibodies to evaluate TRAb profiles after 131I treatment.

Methods: 39 patients diagnosed with GD and treated with 131I were evaluated prospectively. TRAb levels were evaluated before and 1, 2, 3, 6, 9 and 12 months after treatment. TRAb was measured with 2 competitive electro or chemiluminescent assays: Elecsys Anti-TSHR run in Modular E170 (Roche, Mannheim, Germany) and Thyroid Stimulating Immunoglobulins (TSI) run in Immulite 2000 (Siemens, Gwynedd, UK). The first assay measures both stimulatory and inhibitory antibodies, whereas the latter assay detects only stimulating immunoglobulins.

Results: Both assays had a good sensitivity for the diagnosis of GD (Anti-TSHR 100%; TSI 97.4%). Anti-TSHR and TSI profiles were similar after 131I, with a significant rise between the 2nd and 4th month and a progressive decline between the 9th and 12th month. Among 39 patients evaluated, 28 (72%) had an initial TSI increase after 131I. This rise was followed by a progressive decline in 26 patients (93%), but TSI remained higher than basal levels at the end of follow-up in 16 patients (58%). Persistently elevated TSI levels were statistically more frequent in GD patients with ocular disease, longer disease duration and higher TSI at diagnosis. Despite persistent TSI, all patients were cured.

Conclusion: TSI rises after 131I treatment and remains elevated in more than 50% of GD patients up to one year after therapy. These findings suggest that TSI measurements may help in determining when to withdraw antithyroid drug as well as in planning conception in GD women recently treated with 131I who wish to become pregnant.

A-198

Immunoassay Interference in Thyroid Assays: Heterophilic Antibodies

R. Turkal[1], T. Cevlik[1], H. Gozu[2], O. Sirikci[3], G. Haklar[3]. *[1]Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey, [2]Subdepartment of Endocrinology, Department of Internal Medicine, School of Medicine, Marmara University, Istanbul, Turkey, [3]Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey*

Background: Heterophilic antibodies (HA) are endogenous antibodies in human serum/plasma, arising from the natural process of antibody diversity that produces weak, early, multispecific antibodies against diverse antigens. Interference due to HA may lead to falsely low or high analyte levels in a number of immunoassays. Our aim in this study was to investigate the presence of HA as a source of immunoassay interference and possibility of its removal by heterophilic-antibody-blocking tubes in order to correct the discrepancy between obtained thyroid hormon values and the patients' clinical status.

Methods: A total of 41 adult patients referring to the Department of Endocrinology at Ministry of Health-Marmara University Pendik E&R Hospital between November 2017 and January 2019 were enrolled. Selection for the patient group by the Department of Endocrinology was based on clinical-biochemical mismatches including the discrepancies between the laboratory results and the patient's clinical condition, or inconsistent results for thyroid function test (TFT) profile. For screening of HA interference, 500 µL of the serum was added to heterophilic antibody blocking tube (HBT, Scantibodies Laboratories, Santee, CA), gently mixed and incubated for 1 hour at room temperature. The untreated and treated serum were then assayed in the same run on the Beckman Coulter UniCel DxI 800 analyzer for thyroid stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3) and only a significant deviation from the initial result was interpreted as heterophilic interference. In order to determine the significance of the change from the untreated samples, changes were compared to the Total Change Limit (TLC) and Reference Change Value (RCV).

Results: We identified 5 out of 41 patients' samples with suspected interference in fT3 immunoassay. Five patients' serum showed 38.6%, 22.6%, 30.9%, 35.6% and 24.5% decrease of fT3 concentrations upon HBT treatment respectively, whereas no significant difference was observed after the HBT treatment in TSH and fT4 assays. Also, when these five samples were reanalyzed with HBTs, fT3 levels returned within the normal reference range (2.6-4.37ng/L) confirming the presence of HA, except one fT3 sample which was already within the reference range but had a discordantly increased fT4. The 36 samples which did not differ significantly after HBT treatment, showed an absolute difference of 7.89 % (0.2 % - 20.4 %) [mean (range)]; with a 95% CI, 6.04-9.7%. This data may be used as a guide in the evaluation of the significance of the change observed in fT3 results after HBT treatment in the future.

Conclusion: Clinical laboratories should be aware of the possibility of interference by HA and should therefore develop clear analytical and clinical strategies to help identify interference from circulating antibodies to prevent inappropriate management on the basis of erroneous laboratory results.

A-199

A New Fully Automated HbA1c Assay on the DxC 700 AU Clinical Chemistry Analyzers

A. Considine, A. Cahill, L. Frost, S. Frost, S. Hoey, K. Lynch, P. Nolan, D. Shannon. *Beckman Coulter, Co Clare, Ireland*

Background: Diabetes mellitus is a condition characterized by hyperglycemia resulting from the body's inability to use blood glucose for energy. In Type 1 diabetes, the pancreas no longer makes insulin and blood glucose cannot enter the cells to be used for energy. In Type 2 diabetes, the pancreas does not make enough insulin or the body is unable to use insulin correctly. According to the World Health Organization (WHO), 422 million adults were living with diabetes globally in 2014 with an estimated 1.6 million deaths directly associated with diabetes annually. This number of diabetes cases is expected to more than double in the next 25 years. HbA1c is the major species of glycohemoglobin in human blood. HbA1c formation occurs through a non-enzymatic reaction, called glycation, which occurs between glucose and the n-terminal valine of the hemoglobin β-chain of HbA. A Schiff base intermediate product is formed, which rearranges to form a stable ketoamine in an irreversible reaction. The rate of glycation is proportional to the glucose concentration in the bloodstream and is an accurate reflection of average blood glucose over a period of eight to twelve weeks. HbA1c testing is recommended for the diagnosis of diabetes by the International Expert Committee (IEC), the American Diabetes Association (ADA), and the WHO, who recommended a diagnostic threshold of ≥6.5% (≥48 mmoL/moL) HbA1c and a range for pre-diabetes of 5.7%-6.4% (39–46 mmoL/moL) HbA1c. **Methods:** The HbA1c Advanced assay utilizes automatic sample pre-treatment, has batch and random access capability and is FDA cleared on the DxC700AU Clinical Chemistry System. No manual pre-treatment of the whole blood sample or additional washing steps are required. Firstly the red blood cells are hemolyzed automatically, total hemoglobin and glycated hemoglobin are then measured colorimetrically and immunoturbidimetrically respectively. **Results:** Precision studies were conducted according to CLSI EP15-A3. Commercial controls and four native K2 EDTA whole blood samples ranging from 5.1% to 11.7% HbA1c, were run twice daily, over twenty days using three lots of reagent on three DxC700AU Clinical Chemistry analyzers at a single site. Repeatability ranged from 0.72 to 1.12% CV and Total Precision ranged from 1.20 to 2.09% CV. Linearity studies were conducted according to CLSI EP06-A, and verified an analytical measuring range of 4.0 – 15.0% (NGSP) and 20 – 140 mmol/mol (IFCC). Method comparison and bias estimation was evaluated using CLSI EP09-A3. K2 EDTA patient samples (n=138) across the analytical range were run versus a Secondary Reference method and yielded a slope of 0.990, intercept 0.01% HbA1c, correlation coefficient R=0.998 for Weighted Deming regression, and slope 0.980 and intercept 0.09% HbA1c for Passing Bablok regression. Interference studies carried out demonstrated no significant interference from common endogenous interferences, a large panel of drugs, common Hb variants (HbC, HbD, HbE, HbA2 and HbS) and cross reactants (HbA0, HbA1a+b, acetylated hemoglobin, carbamylated hemoglobin, glycated hemoglobin, glycated albumin and labile HbA1c). **Conclusion:** The HbA1c Advanced assay on the DxC700AU is a precise and accurate assay, requiring no manual pretreatment and can be used for monitoring and diagnosing diabetes.

A-200

Minor Peak Elevations of the P3 Rraction in the D-100™ Bio-Rad HPLC System are not Explained by Hemoglobin Variants or Hemoglobin Degradation

C. Kovacic, Y. Zheng, M. McCale, J. Potz, S. Ondrejka, J. M. Colon-Franco. *Cleveland Clinic, Cleveland, OH*

Background: Hemoglobin A1c (HbA1c), the major form of glycated hemoglobin, is the gold standard for long-term monitoring of glycaemia and for diabetes diagnosis. HbA1c results from the non-enzymatic binding of glucose to the N-terminal region of hemoglobin beta chains. The intermediate labile A1c (LA1c) is characterized by reversible binding of glucose to hemoglobin. When evaluating HbA1c by ion-exchange HPLC, normal and abnormal peaks, such as hemoglobin variants, are detected. A typical chromatogram in the D-100 ™ system (Bio-Rad) has seven peaks, eluting as follows: HbA1a, HbA1b, HbF, LA1c, HbA1c, P3, and HbA0. HbA1c is calculated from the ratio of HbA1c to all HbA-derived fractions. Hemoglobin fractions eluting after the HbA0 peak are excluded, but hemoglobins eluting near the HbA-derived fractions could interfere. The P3 peak represents degraded HbA0 and peak areas greater than 10% are considered indicative of hemoglobin variants or sample degradation. Hemoglobin variants known to co-elute in the P3-window include Hb Camden, Hb Hope, Hb J Oxford, Hb Austin, Hb N-Baltimore and usually represent 20-45 % of total hemoglobin. A P3 peak area >10% flags in the D-100 system as 'minor peaks' for evaluation of possible hemoglobin interference. We noticed that samples with P3 10-20% often had concurrent elevated HbA1c which correlated clinically.

Methods: We investigated if P3 peaks 10-20% indicated the presence of hemoglobin variants in consecutive samples with P3 10-20% from January - February 2019. The Sebia CapiFlex system was used for hemoglobin evaluation. We studied the effect of sample storage at room temperature (n=20) or refrigerated (n=20) in the P3 area after storage for 3 and 5 days using left-over samples with A1c ranging from 4.4-11.1%.

Results: Five patient samples with P3 areas from 10.02-19.34% flagged for review. A1c in these samples ranged from 7.4-14.7%. None had hemoglobin variants identified. Pre-analytical issues remain unidentified. A sample with a peak area of 44.58% in the P3-window also flagged for review, which was characterized as Hb J-Baltimore. In the samples used for stability studies, P3 ranged from 4.22-7.03% of total hemoglobin. A1c correlated with P3 and LA1c (r 0.75 and 0.7, respectively) (n=40). The average bias (%bias) after refrigeration for 5 days was 0.1 (1.5%) for HbA1c and -0.2 (-4.2%) for P3. Storage at room temperature showed an average bias (%bias) of 0.1 (1.4%) for HbA1c and -0.5 (-8.1%) for P3 after 3 days and of 0.1 (1.1%) for HbA1c and 0.6 (12.2%) for P3 after 5 days.

Conclusion: Despite P3 increasing after storage at room temperature for 5 days, none of the samples resulted in P3 areas >10%. As described elsewhere, a subset of cases with elevated P3 actually do have variants, particularly those with P3 areas >20%. While some samples with a P3 fraction of 10-20% may express Hb variants or degradation products, in our study, minor P3 peaks were not explained by Hb variants, temperature, or storage. Hemoglobin evaluation in a larger cohort of samples with minor P3 peaks is needed to further characterize the significance of these elevations.

A-201

Immunoassay Performance for Thyroid Function Analytes on the Roche Cobas® Platform during Pregnancy

K. A. Geno[1], M. S. Reed[2]. [1]*Dartmouth-Hitchcock Medical Center, Lebanon, NH,* [2]*Southern New Hampshire University, Manchester, NH*

Background: Thyroid disease during pregnancy increases the likelihood of fetal and maternal comorbities. Automated FT4 immunoassays are widely-utilized in hospital-based laboratories; however, changes in thyroid hormone binding capacity during pregnancy can affect their accuracy. The FT4 immunoassay used in our laboratory was reported to generate falsely-low values in the second and third trimesters; however, subjects were limited in number and of a different racial demographic than our patient population. Additionally, the package insert for our T4-uptake (TUP) assay does not indicate pregnancy-specific reference intervals despite the known increase in thyroxine-binding globulin throughout pregnancy. We sought to verify the performance of our thyroid immunoassays during pregnancy. **Methods:** We collected remnant specimens from pregnant women and nonpregnant women of childbearing age (18-45 years) and excluded specimens with anti-thyroid antibody concentrations above our reference limits. For remaining specimens (minimum 120 per trimester and 120 nonpregnant controls [NPCs]), we measured hCG, total T4, FT4, TSH, and TUP on the Roche Cobas®. **Results:** Consistent with expected increases in thyroxine-binding globulin, TUP increased throughout pregnancy. The manufacturer-provided reference interval excluded greater than 65% of second trimester (Tri2) and 88% (Tri3) of specimens. Concordant with a previous study, FT4 did not change significantly between NPCs and Tri1 patients; however, median FT4 was less than 15% lower in Tri3 than in NPCs and less than 10% lower in Tri2. FT4 correlated well with FT4 index, which has been proposed as a more reliable alternative to FT4. **Conclusion:** Laboratories should generate trimester-specific TUP reference intervals for their patient populations. Although mean FT4 values did vary in a statistically-significant manner throughout pregnancy, the number of specimens outside the manufacturer's reference interval was not significant until Tri3, when a separate interval may be justified. Future studies will be needed to determine the effectiveness of trimester-specific reference intervals in detecting thyroid disease.

Selected Thyroid Function Analytes, Median by Trimester						
	T4 Uptake		FT4, This Study		FT4, Previous report[1]	
	Median (95% CI), Ratio	% Change vs. NP[2]	Median (95% CI), ng/dL	% Change vs. NP	Median (95% CI), ng/dL	% Change vs. NP
Non-pregnant	1.07 (1.05-1.09)	-	1.24 (1.20-1.29)	-	1.10 (0.90-1.40)	-
1st Trimester	1.18 (1.16-1.21)	+10.3	1.24 (1.20-1.29)	-0.4	1.20 (0.90-1.62)	+9.1
2nd Trimester	1.34 (1.31-1.37)	+24.8	1.13 (1.08-1.17)	-8.9	0.85 (0.66-1.08)	-22.7
3rd Trimester	1.40 (1.38-1.42)	+30.8	1.06 (1.02-1.09)	-14.5	0.89 (0.64-1.13)	-19.1
[1]Lee RH et al. (2009) Am J Obstet Gynecol 200:260.e1; [2]Nonpregnant						

A-202

Modeling of Relationship between Glycated Albumin and Glucose: Characterization and Comparison to Hemoglobin A1c

D. F. Stickle. *Jefferson University Hospitals, Philadelphia, PA*

BACKGROUND: Glycated albumin (GA) is recommended for assessment of glycemic control in circumstances in which measurement of hemoglobin A1c may be unreliable. Glycation of albumin at the molecular level is known to be mechanistically parallel to the process of glycation of hemoglobin. We used this model to characterize the predicted relationship between average glycated albumin and glucose from a theoretical standpoint. **METHODS**: As for hemoglobin, glycation of albumin at the molecular level involves formation of an unstable intermediate form of glycation that can convert to a stable, largely irreversible form. Glycation of albumin can occur at multiple sites. Corresponding to prior models for A1c, the probability (P) of glycation of an albumin molecule at a given time (t) was treated as a function of glucose (G) and a composite rate constant for conversion (k); for constant G, the glycation equation is of the form P(t)=1-exp(-k [G] t). The total GA fraction was given by GA=sum([P](t) x f(t)), where P(t) is the probability of glycation as a function of age of the molecule, and f(t) is the fraction of molecules in blood having that age. f(t) in increments of 1 day for albumin was derived from the survival curve (S(t)) for albumin, which was treated in textbook fashion as a first-order decay process with a half-life of 17 days: S(t)=exp(-t/τ), τ=24.5 days. Determination of k was by fitting of predicted GA as a function of glucose to data of Chen et al. [*Clin Chem* 2017, PMID 27737895] for nonuremic subjects across the range of 5-11 A1c% (estimated average glucose (eAG) in range of 5-15 mmol/L). **RESULTS**: The value of k=0.00104/day/(mmol/L) was a best fit approximation to experimental data of Chen et al. for GA vs. eAG in the range of 5-15 mmol/L (r2=0.998). Although not formally linear, GA across this range for G had a high linear correlation coefficient (GA(%)=1.59 G (mmol/L)+3.06; r2=0.998). There was close numerical correspondence of predicted GA with reference range data for GA from Selvin et al. [*Clin Chem* 2018, PMID 29436378] at cutpoints associated with the upper limit of the refence range for A1c (5.7%) and with diabetes (6.5%): predicted GA=13.2% vs. 13.6% for A1c=5.7% (eAG=6.5 mmol/L); predicted GA=15.5% vs. 15.6% for A1c=6.5% (eAG=7.8 mmol/L). In increments of days, the albumin age fraction having the greatest incremental contribution to total GA was at 23 days. Simulations of postprandial elevations in G produced no changes in GA when daily average glucose was maintained. GA is thus not predicted to be a specific indicator of postprandial G excursions in comparison to A1c, corresponding to conclusions of recent clinical trial data analysis by Paul & Holman [*Diabet Med* 2017, PMID 28477414]. **CONCLUSIONS**: Modeling of kinetics of formation of glycated albumin as a function of glucose, parallel to established models for A1c, produced results that were in close correspondence to experimental data. Utility of GA should be thought of in the same, simple general terms as A1c, that changes reflect changes in average glucose, but on a considerably fore-shortened (1-month) time scale.

A-203

Vitamina D Intoxication: Impact of Megadose Therapy

C. O. W. Sabino, D. Waltrick, L. Rodrigues, L. B. Avallone, G. A. Campana. *DASA, São Paulo, Brazil*

Background: Vitamin D intoxication is characterized by the excess of this vitamin that leads to hypercalcemia, which can cause nonspecific symptoms, such as anorexia, weight loss and polyuria or more serious complications such as cardiac arrhythmias, vascular and tissue calcification, and can lead to kidney failure. In the literature, a serum concentration of 25(OH)D consistently >500 nmol/L (> 200 ng/mL) is considered potentially toxic. The toxicity is much more likely to occur from the intake of supplements containing vitamin D. From the observation of the increase of intoxication cases by Vitamin D in the routine of a mega Brazilian Laboratory, the objective of this study was to verify this incidence. **Methods:** Routine data from the years of 2012, 2017 and 2018 were analyzed, considering various parameters involved in calcium metabolism, such as total and ionized calcium, PTH (parathyroid hormone), 25(OH) vitamin D and creatinine. The methodology used for 25(OH)vitamin D and PTH assays was the chemiluminescence in ADVIA Centaur XPT, CPC for total calcium in ADVIA Chemistry, Jaffe for creatinine in ADVIA Chemistry and selective ions by the AVL analyzer for the ionized calcium assay. **Results:** In a comparative analysis between October 2012, 2017 and 2018, although the number of hypercalcemia due to all causes remained relatively stable, with a decrease in 2017 (7.9% to 4.9%) and a slight increase in 2018 of 5.53%, those related to hypervitaminosis D increased from 0.27% in 2012 to 0.82% in 2017 and in 2018 a significant increase of 1.71%, with a total growth of 6.3% in cases of intoxication over the last 6 years. Cases of vitamin D intoxication were observed with concentrations starting at 102.66 ng/mL. **Conclusion:** The incidence of vitamin D intoxication has doubled from 2017 to 2018, suggesting that the prescription of vitamin D megadoses remains a practice in therapeutic protocols as a treatment for autoimmune diseases, and monitoring therapy with only serum dosages of calcium and vitamin D is not effective. The literature suggests monitoring for the prevention of hypercalcemia is the measure of calcium in urine for 24 hours. It is important for the medical society to be alerted to the risks of intoxication associated with megadoses of vitamin D and how therapy should be controlled. **Siemens Healthineers supported the studies by providing systems and reagents.*

A-204

Performance Evaluation of a Parathyroid Hormone (PTH) In Vitro Diagnostic Immunoassay on a Novel Point of Care Device

C. Rosmalen. *NBCL B.V., Nijmegen, Netherlands*

Background: Current Point of Care (PoC) immunoassay testing often lacks sensitivity and precision in comparison to results generated by the central laboratory. Diagnostics in central laboratories causes delayed test results, through logistics and availability. This increases the need for a PoC platform with rapid and accurate results, to improve efficiency and effectiveness of clinical care, especially in the operating and emergency room. Here, the proof of principle of a proprietary PoC platform measuring PTH in whole blood during parathyroidectomy in patients with primary hyperparathyroidism is described. This is done in order to improve the success rate of the surgery. The PoC platform with the PTH immunoassay has a high sensitivity and precision and time to result is only 5 minutes.

Methods: Paramagnetic particles were coated with an anti-PTH antibody directed to the C-terminal part of PTH. The particles were incubated with sample (whole blood/ serum/ plasma), assay diluent, and an anti-PTH antibody (raised against the N-terminal part of PTH) conjugated with alkaline phosphatase. Incubation temperature was 37°C and incubation time was 90 seconds. After washing the sandwich assay using a magnet a chemiluminescent substrate was added, generating a glow signal. The relative light unit is measured using a photomultiplier and the amount of light correlates with the amount of PTH in the sample.

Results: The Limit of detection on the PoC device is below 1.0 pg/mL. Correlation with a predicate device (n=35) resulted in a correlation coefficient of 0.90, a slope of 1.14 and an intercept of -3.6. A comparison in results between whole blood and EDTA plasma samples on the PoC device resulted in a correlation coefficient of 0.93, a slope of 0.96, and an intercept of 3.0. Testing the novel PoC device with the PTH immunoassay during surgery in patients with primary hyperparathyroidism showed promising results; from eighteen patients the PTH assay positively confirmed removal of the adenoma by the surgeon. Successful removal of the adenoma results in a 50% drop of the PTH level in 5 - 10 minutes after resection of the suspected adenoma, compared with the level prior to surgery.

Conclusion: The quick PTH immunoassay on the novel PoC device demonstrated good performance during surgery in patients with primary hyperparathyroidism. Furthermore, good correlation and agreement with a predicate device was observed.

A-205

Establishment of Gestational Stage-Specific Reference Ranges for TSH in Pregnant Turkish Woman: A Preliminary Report

S. Aytan[1], B. Cakmak[1], B. Gozaydınoglu[1], C. Takır[1], C. Turan[2], O. Unlu[3], G. Bahramzada[3], A. Tekin[3], T. Cevlik[4], H. Gozu[5], O. Sirikci[3], G. Haklar[3]. *[1]Medical Student, School of Marmara University, Istanbul, Turkey, [2]Department of Internal Medicine, School of Medicine, Marmara University, Istanbul, Turkey, [3]Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey, [4]Biochemistry Laboratory, Marmara University Pendik Education & Researh Hospital, Istanbul, Turkey, [5]Subdepartment of Endocrinology, Department of Internal Medicine, School of Medicine, Marmara University, Istanbul, Turkey*

Background: Pregnancy has a profound impact on thyroid function as human chorionic gonadotropin (hCG) and thyroxine-binding globulin (TBG) levels change which affect hypothalamic-hypophyseal axis, TSH, and free thyroxine concentrations. For these reasons, thyroid function is frequently assessed during the pregnancy, but their interpretation is difficult. According to American Thyroid Association guideline, trimester based TSH limits are 0.1-2.5 mU/L, 0.2-3 mU/L, and 0.3-3 mU/L for the 1st, 2nd, and 3rd trimesters, respectively. However, the concentration of TSH also changes within trimesters due to abrupt changes in hCG and TBG concentrations. Our aim was to calculate gestational stage-specific TSH reference ranges and compare the diagnostic value of fixed cut-off values specific for trimesters.

Methods: A total of 116 pregnant women with no known thyroid disease, optimal iodine intake, and negative TPOAb status who underwent routine prenatal care from January 2017 to January 2018 at Marmara University Pendik E&R Hospital were enrolled in the study. Fasting blood samples were collected into gel separator tubes (BD, USA). TSH levels were measured by DxI 800 Immunoassay System (Beckman Coulter, USA). TSH values were grouped as T1-1 (4-8 wks, n=21), T1-2 (8+1day-12 wks, n=24), T2-1 (12+1day-20 wks, n=39), T2-2 (20+1day-27wks, n=20), T3-1 (27+1 day-33 wks, n=6), and T3-2 (33+1 day to 40 wks, n=6). The data were analyzed by SPSS 18.0 version. The gestational stage-specific data were expressed as [median (2.5th-97.5th percentiles)]. **Results:** The median (2.5-97.5 percentiles) for groups were: T1-1 [1.1(0.22-3.45)], T1-2 [1.1(0.16-4.89)], T2-1 [1.57 (0.34-3.76)], T2-2 [1.74 (0.75-5.51)], T3-1 [1(0.43-1.68)], and T3-2 [1.65(0.17-3.02)] mU/L. According to trimester based TSH levels 14 patients were diagnosed to have hypothyroidism whereas only 5 of them were above gestational stage-specific refence ranges. **Conclusion:** When possible, population-based reference ranges for serum TSH should be determined for the assessment of local data

A-206

Insulin Signaling Activation in Skeletal Muscle and Upregulated Ceramide *De Novo* Synthesis

P. A. Ramos, K. Lytle, M. Jensen. *Mayo Clinic, Rochester, MN*

Background: Insulin resistance occurs in ~25% of the human population, being this condition a chief component of Type 2 Diabetes. It is known that high Free Fatty acids (FFA) in plasma negatively impact muscle glucose disposal; however, the mechanisms behind this are still not fully understood especially in humans. Previous reports have indicated that intramyocellular ceramides may be involved in pathological metabolic stresses which could negatively impact AKT activation and phosphorylation—a major regulatory step in the insulin-signaling cascade. We hypothesized that a surplus of FFA in plasma incorporate into ceramides in the skeletal muscle becoming toxic and interfering with regular glucose uptake. **Methods:** 14 Lean and 14 obese men and women underwent muscle biopsies before and at the end of a single step hyperinsulemic-euglycemic clamp. A [U-^{13}C]palmitate infusion was started 5-h prior to the clamp and replaced with a 5-h infusion of [$^2H_9$]palmitate during the clamp to assess the contribution of plasma FFA to de novo C16-ceramide synthesis under basal and hyperinsulinemic conditions, respectively in total, subsarcolemmal (SS), and intramyofibrillar (IMF) portions. We measured phospho-Ser473 and total Akt, AS160 (the next Akt downstream effector for glucose uptake) as well as total GLUT4 content using a capillary Western blot approach. Ceramide species concentration and isotopic enrichment were measured with LC/MS/MS. Insulin sensitivity was calculated as the glucose disposal rate over final hour of the insulin clamp adjusted per kg fat free mass as a function of plasma insulin concentrations. **Results:** The increases in Akt and AS160 phosphorylation were not different in lean and obese subjects despite a much lower glucose disposal rate. Total muscle GLUT4 protein content averaged 12% less in obese than lean participants ($p<0.05$). We found that a greater contribution of plasma palmitate to de novo synthesized C16:0-ceramides during the insulin clamp in the obese group within the total and IMF fraction was negatively correlated with the fold increase in Akt phosphorylation ($P < 0.05$, -r= 0.91/ $P < 0.05$, -r = 0.88) and negatively correlated with glucose uptake ($P < 0.01$, -r =0.92/ $P < 0.05$, -r = 0.68). **Conclusion:** These finding suggest that newly synthesized ceramides from plasma FFA are negatively related to glucose uptake and activation of AKT downstream effectors of the insulin signaling pathway. Filling in the gaps of knowledge that exist in this area is essential to understanding the underlying mechanism of IR development in order to seek new therapeutic options.

A-207

Study to Reduce Test Repetition in ROCHE Equipments (COBAS 8000)

A. C. M. Wajdowicz, G. M. de Abreu, G. Grejianin, L. Rodrigues, A. Martins. *DASA, Barueri, Brazil*

Background: A common practice in clinical laboratory analysis has been use computerized system for tests repetition when values do not satisfy certain pre-established conditions in the laboratory system - considered normal concentration ranges. The objective of the study was to establish criteria that would lead to a reduction in tests repetitions without reducing reports reliability.

Methods: We retrospectively evaluated the database of TSH dosages performed between September 2017 and February 2018 performed on the COBAS8000 equipments. All replicates were tabulated in order to evaluate the repetition rules adopted by the laboratory. As a premise, the coefficient of variation between the first measure and the repetition was used, compared to coefficient of intra-individual variation (CVi), in other words, variation of the results from sample in the same examination belonging to a single individual.

Results: There were 1 218 260 TSH measurements in the period, among which 73 306 (6%) were repetitions. For results below 0.1 µIU/m, was observed need for repetition due to the possible presence of bubbles and fibrins in the sample; in results above 100 µUI/m dilution becomes necessary because of tests linearity used. In reference range considered normal for TSH (0.4 to 5.5 µUI/m) repetition was proved to be unnecessary, since results were confirmed in 99.61%. For 74 replicates (0.39%) that did not present satisfactory CVi in this range when compared to the tabulated value by Westgard (2014) the repetition was considered unnecessary according to clinical criteria, technical by bibliographic basis, such as preanalytical interferences, clinical conditions of the patient stability, sample stability, and CV sensitivity for small value ranges and similar clinical reports.

Conclusion: Starting from the range of reduction proposed in the work, it is estimated a reduction of 19,150 repetitions, representing a decrease of 26% compared from a total of 73,306, bringing benefits such as increase of productive capacity, reduction of wastes and narrowing total service time . In addition, this rules described in this article can be applied in other tests in the normal ranges of reference and for this it is necessary to extend this study. It is worth noting that errors in laboratory results are more often related to pre-analytical and post-analytical factors than to analytical errors. Analytic issues account for only 8% to 15% of clinical errors, while errors related to pre-analytical and post-analytical questions account for 85% to 92% of all errors.

A-208

Total and Free 25 Hydroxy Vitamin D are Associated with AMH and Androgens in Women with Biochemical Evidence of Polycystic Ovary Morphology

M. M. Kushnir[1], V. A. Kushnir[2], J. A. Straseski[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]Wyckoff Heights Medical Center, Brooklyn, NY, [3]University of Utah School of Medicine, Salt Lake City, UT*

Background: Vitamin D (VITD) has been associated with numerous physiologic functions and conditions, including fertility and polycystic ovary syndrome (PCOS). Prior studies differ on whether an association exists between total 25 hydroxy vitamin D (T25OHD) and anti-Mullerian hormone (AMH) and no studies have evaluated the association between AMH or androgens and the proposed biologically active form of 25OHD, free 25OHD (F25OHD). Measurement of F25OHD could be a better indicator of VITD sufficiency, particularly in patients with disorders affecting binding

protein synthesis. We investigated these relationships in reproductive-aged women (RAW) with and without biochemical evidence of PCO morphology (PCOM).

Methods: We evaluated the association of AMH with T25OHD, F25OHD, and androgens (testosterone (Te), dehydroepiandrosterone (DHEA) and androstenedione (A4)) in reproductive-aged women (RAW; n=121; mean age (range) 28.9 years (18-39); SD 6.3). T25OHD3, F25OHD2, F25OHD3 and androgen concentrations were measured using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods, including a recently developed F25OHD method. AMH was measured using ELISA (Ansh Labs). Total imprecision of all methods was <15%. VITD deficiency (sufficiency) was defined as T25OHD <20 (>30) ng/mL and F25OHD <0.024 (>0.04) ng/mL. Androgen excess was defined as DHEA >7.8 ng/mL, A4 >2.14 ng/mL, and/or Te >0.55 ng/mL (Kushnir et al., Clin Chem, 2010). Biochemical evidence of PCOM was defined as AMH concentrations >4.7 ng/mL (Iliodromiti et al., JCEM, 2013) and/or at least one of the androgens above the respective reference interval (RI) (n=51).

Results: Statistically significantly higher AMH concentrations were observed in VITD insufficient and deficient (based on T25OHD) RAW (p=0.023), while no significant difference was observed when VITD status was defined based on F25OHD. In women from the PCOM

group, DHEA, A4 and Te concentrations were statistically significantly lower in women with T25OHD deficiency (p=0.033, 0.013 and 0.019, respectively) and F25OHD deficiency (p=0.0059, 0.0072 and 0.015, respectively). No statistically significant association between androgen concentrations and VITD status (free or total) was observed in the non-PCOM group. T25OHD concentrations were significantly lower in the PCOM group (p=0.05) compared to non-PCOM, while concentrations of F25OHD were not statistically different between the groups (p=0.76). However, statistically significantly higher percent F25OHD (p=0.029) was observed in the PCOM group. F25OHD correlated with T25OHD in both PCOM and non-PCOM groups. DHEA concentrations were statistically significantly higher (p=0.0051) and A4/DHEA ratios were lower (p=0.023) in samples with AMH >4.7 ng/mL.

Conclusions: Our data indicated that elevated AMH is associated with VITD insufficiency in RAW and higher concentrations of androgens (DHEA A4 and Te) are associated with VITD above the deficiency cutoffs in women with biochemical evidence of PCOM. Since lower T25OHD would normally associate with lower F25OHD, the elevated F25OHD fraction in women with biochemical evidence of PCOM is likely explained by the lower concentrations of binding proteins found in this condition. Associations of F25OHD with AMH, T25OHD, and androgens have not been described previously, particularly in PCOM populations, and may provide a new area of investigation.

A-209

Fasting flexiblization for the Gh Post-Clonidine Test: - Impact on Side Effects and on GH Response to the Stimulus

Y. Schrank, A. F. D. F. Pedrozo, G. R. Ramos, E. M. R. Cavalari, P. M. C. Araújo, M. D. Freire, G. A. Campana, M. M. C. Pinheiro, D. V. Gomes, R. Fontes. *DASA, Rio de Janeiro, Brazil*

Background: The growth hormone (GH) stimulation test with clonidine is a frequently requested test in the routine of short stature investigation in childhood. Clonidine is an α2 adrenergic agonist drug, that promotes release of GH releasing hormone (GHRH). It has as common side effects drowsiness, bradycardia and hypotension. Orthostatic hypotension, with the presence of symptoms and requirement of volume replacement can be considered a severe side effect. The effect of fasting on the absorption and action of clonidine is poorly defined in the literature. It is known that after oral administration, clonidine is rapidly absorbed, between 20 and 30 minutes, with an average bioavailability of 55.4% in children (47% to 65%), reaching maximum serum level within 60 minutes. **Objective:** To evaluate the impact of fasting flexibilization for the GH stimulation test with clonidine in terms of severe side effect and GH response to the stimulus. **Design:** The present study was a retrospective cohort study. **Methods:** We performed a retrospective analysis of children submitted to the GH stimulation test with clonidine in a laboratory reference center over a 5-year period. We divided the children into 2 groups: *Group 1* (***Absolute Fasting Group***): children undergoing the GH stimulus test who fasted throughout all the test (feeding only after the last sampling) and *Group 2* (***Flexibilized Fasting Group***): children undergoing the test after fasting for 8 hours prior to the start, but feeding after 30 minutes of clonidine administration. Normal GH response to clonidine was defined as a GH peak >= 5.0 ng/mL. Severe side were defined as presence of symptomatic hypotension (dizziness, fainting) with requirement of ethylefrine chloridrate or even volume replacement for symptom reversal. **Results:** Three hundred and forty-eight patients were eligible for the study, being 142 patients in group 1 (***Absolute Fasting Group***) and 206 in group 2 (***Flexibilized Fasting Group***). **GH response:** 88.7% patients in group 1 were responsive to the stimulus versus 85.9% patients in group 2, which was not statistically significant (p = 0.4413). Mean GH peak, respectively, at baseline, 60 and 90 minutes after clonidine was 0.74, 9.79 and 8.82 ng/mL in group 1 versus 0.99, 7.59, 8.02 and ng/mL in group 2. Comparative analysis between the groups, again, showed no difference between GH responses (p was respectively 0.6, 0.8 and 0.8 for each compared sampling time). **Side effects:** Because of symptomatic hypotension ethylefrine was administered at the end of the test to virtually all patients in group 1, on the other hand, no special management had to be adopted for patients in group 2. Comparing patients of either group who did not receive ethylefrine, 8.7% patients of group 1 and 2.9% patients of group 2 required volume expansion, which was statistically significant (p < 0.05). **Conclusion:** Based on our data, we conclude that fasting flexibilization appears to be an interesting feature in the evaluation of patients undergoing GH stimulation with cloinidine. The procedure resulted in a significant decrease in adverse side effects with no interference in the magnitude of GH response to the stimulus.

A-210

Heterogeneity of Macroprolactin in Samples Suspected of False Hyperprolactinemia

K. Nguyen[1], R. Langevin[1], M. J. McPhaul[2], I. A. Hashim[1]. [1]*University of Texas Southwestern Medical Center, Dallas, TX,* [2]*Nichols Institute, San Juan Capistrano, CA*

Background:

Circulating prolactin (PRL) exists in several molecular forms with different biological and immunological activities, in some instances making PRL measurements and its diagnostic value unreliable. Macroprolactin (macroPRL), a 150 kDa molecular weight (MW) form, is known to have low biological activity which may lead to misdiagnosis and inappropriate treatment in patients with suspected hyperprolactinemia. Polyethylene glycol precipitation (PEGP) is widely used to identify the presence of macroPRL, but up to 20% of monomeric PRL (23 kDa) is lost during this procedure and PEG itself can interfere with some immunoassays. Additionally, PEGP has been reported to give false positive results for macroPRL in patients with increased serum globulins (IgG myeloma and HIV patients). The aim of this study is to identify different PRL variants in samples suspected of macroPRL using gel filtration chromatography (GFC).

Methods:

Thirteen samples suspected of having macroPRL (as determined by PEG) were obtained from a collaborating reference laboratory. Briefly, 100 uL of sample or protein markers (MWs ranging from 12.4 to 200 kDa) were applied to a Superdex 200 column (Pharmacia). Forty fractions of 0.5 mL each were collected per patient sample at a flow rate of 0.4 mL/min using PBS containing 0.1% (w/v) bovine serum albumin as the mobile phase. Elution of marker proteins was detected by recording absorbance at 280 nm. To pull down PRL-IgG complexes from our samples, 100 uL of each sample was applied to NAb protein G spin columns (Thermo Scientific) according to the manufacture's protocol. The elution and flow-through fractions were collected and analyzed by GFC as described above. Five samples containing non-IgG-bound macroPRL were selected after GFC results, and glycosylated variants in those samples were examined using Concanavalin-A lectin columns (GE Healthcare, USA). Briefly, 500 ul sample was applied to 1 mL column and bound prolactin was eluted using 0.5M methyl-alpha-D-glucopyranoside. All PRL concentrations were measured using ELISA (RnD systems) according to the manufacture's protocol.

Results:

PEGP suggested 8 of our 13 samples were positive for macroPRL, 3 samples had no macroPRL, and 2 samples are within the indeterminate zone (recovery between 40-60%). GFC analyses corelated well with those results, and no macroPRL was present in samples from the indeterminate zone according to GFC. In samples containing macroPRL, 30.8 - 99% of total PRL was bound to protein G columns, while only 0 - 4% of PRL was pulled down from samples negative for macroPRL. Of 8 samples positive for macroPRL, 5 had a fraction of macroPRL that was not bound to protein G columns suggesting the existence of non-IgG-bound macroPRL. Also, in those 5 samples, the percentage of total glycosylated prolactin variants ranged from 1.7% to 6.7%.

Conclusion:

GFC analysis showed marked molecular heterogeneity for macroPRL. Mid-molecular weight-PRLs (30-150kDa) were present in every sample, and both high-molecular weight-PRL (>150kDa) and mid-MW-PRL had diverse patterns, but their clinical significance and physiological roles remained unclear. This is the first study to report molecular heterogeneity for macroPRL.

A-211

Hyperthyroidism Diagnostic Test Utilization: An Evaluation of Current Ordering Practices

G. Altawallbeh, J. W. Rudolf, A. B. Karger. *University of Minnesota, Minneapolis, MN*

Background: In 2011, the American Association of Clinical Endocrinologists (AACE) and American Thyroid Association (ATA) issued a joint guideline on hyperthyroidism diagnosis recommending radioactive iodine uptake (RAIU) as the test of choice when the clinical presentation for thyrotoxicosis is not diagnostic for Graves' Disease. The guidelines state that thyroid receptor antibody (TRAb) testing should only be utilized when RAIU +/- thyroid scan is unavailable or contraindicated. In 2016, the ATA published updated guidelines that shift to recommending TRAb testing over RAIU, due to cost effectiveness; the AACE has not published new guidelines since 2011.Given the current discrepancy between the latest AACE and ATA guidelines, we aim to evaluate our institution's current diagnostic test ordering practices for hyperthyroidism. **Methods:** Thyroid diagnostic test data from October to December 2017 was extracted and analyzed from the electronic medical records of patients seen at Fairview Health Services. The diagnostic tests of concern included RAIU, and two assays for measuring TRAb - the TSH binding inhibition immunoglobulin (TBII) assay, and thyroid stimulating immunoglobulin (TSI) bioassay. **Results:** During the study period there were 251 patients with 310 individual diagnostic thyroid test orders. The most commonly ordered test was TSI (n = 226, 73%), followed by RAIU (n = 48, 15%) and TBII (n = 36, 12%). TSI and RAIU were both ordered at the same visit for 15 patients, with results showing 100% agreement. In 23 cases, TSI and RAIU were ordered on the same patient at separate visits, with 18/23 showing concordant results. Five patients had TBII and RAIU ordered at sequential visits, with 100% concordance. Two patients had all three tests ordered (TSI, TBII and RAIU) with 100% concordance. **Conclusion:** At our institution, providers' clinical practice reflects the more recent 2016 ATA guidelines rather than the 2011 AACE guidelines, with reliance on TRAb rather than RAIU for initial diagnostic testing in patients with thyrotoxicosis. Among the two options for TRAb testing, our providers primarily utilize TSI in the initial workup of hyperthyroidism even though it has a longer turnaround time (4 days vs. 2 days), is ~1.5 times more expensive, and has greater analytical variability than TBII. Although the TSI assay is considered the gold-standard TRAb test, studies have demonstrated comparable sensitivity and specificity with TBII, and thus encouraging our providers to utilize TBII testing has the potential to increase cost savings and decrease turnaround times.

A-212

ID-LC/MS-MS Reference Measurement Method for Cortisol in Serum

D. Ducroq, G. L. Brett, M. A. Thomas. *Weqas, Cardiff, United Kingdom*

The traceability of laboratory results is a recognised need within quality standards based on ISO 15189 and ISO 17025. Where possible, higher order reference measurement methods are required to provide traceability to the SI unit for vitro diagnostic measurement results, ensuring the transfer of accuracy from definitive methods to routine methods.

A reference measurement method for cortisol based on a published method using exact matching isotope dilution LC-MS/MS has been developed and validated within the Weqas Reference Measurement Laboratory. Traceability was assured by the use of NIST 921, with an accuracy matrix check using ERM-DA192 and ERM-DA193.

Sample preparation involved solid phase extraction with Isolute® columns. Reconstituted samples were ionised by Electrospray Ionisation in positive mode. These ions were then analysed by mass spectrometry in mixed reaction mode (MRM), monitoring ions m/z 363.3 (cortisol P) and m/z 366.3 ($^{13}C_3$-cortisol P) for each sample, control and standard. The ratio of this ion pair was converted to a cortisol concentration via reference to bracketed standard curves. An MS scan was also carried out during analysis, to detect any contaminants.

No major interferents were identified during the validation phase. The maximum imprecision was within published reference measurement criteria based on duplicate analysis of samples on 3 separate occasions. Bias of the reference material was also within accepted criteria for a mass spectrometer method for cortisol. Good performance of the method was observed with the RELA external quality control programme for Reference Laboratories.

This reference measurement method has been used to assess the performance of routine cortisol methods for all distributed samples within the Weqas Proficiency Testing Endocrine programme. Reference measurement data is useful as an accuracy target in Proficiency Testing Programmes.

A-213

Review of Thyroid Function Test Ordering Patterns in Routine Pregnancy Across Two Academic Medical Centers

K. Hardeman[1], T. Osborne[1], J. Colby[2], L. Lee[2], B. Adams[2], A. Woodworth[1]. [1]*University of Kentucky Medical Center, Lexington, KY,* [2]*Vanderbilt University Medical Center, Nashville, TN*

Background: Maternal thyroid dysfunction during pregnancy can lead to adverse effects on the fetus, including developmental delay, premature birth and miscarriage. Trimester specific reference intervals and evidence based laboratory testing algorithms are important for guiding the diagnostic work up of thyroid dysfunction screening in pregnancy.

Objective: The aim of this study was to review the ordering practices of obstetricians and midwives and make recommendations to reduce inappropriate ordering and misinterpretation of thyroid function testing in pregnancy.

Methods: Records from all routine prenatal patient visits from two large academic medical centers (University of Kentucky Medical Center and Vanderbilt University Medical Center) for distinct 6 months and 1 year time periods respectively, were retrieved utilizing ICD9 and 10 codes representing normal pregnancies on a day which syphilis screening was performed. Patients were excluded if the pregnancy was not singleton, the patient was <18 years of age, and/or had a history of thyroid disease, type 1 diabetes, other autoimmune disorders or a malignant neoplasm. Additionally, we verified trimester specific reference intervals (TSRI) for TSH and FT4 at each institution using their respective analyzer platforms (Roche Cobas and Abbott Architect). Thyroid function test results were considered in the context of institution and trimester specific reference intervals. Patients' electronic medical records were reviewed to determine follow up to thyroid function testing and pregnancy outcomes.

Results: In total, we evaluated 1787 pregnancies from VUMC and 2531 pregnancies from UKMC. After applying exclusion criteria, we were left with 1672 and 2075 healthy adult singleton pregnancies, respectively. TSH was ordered for 1077 of 1672 pregnancies at VUMC, and 430 of 2075 pregnancies at UKMC. Order set and chart review of patients suggested that thyroid test ordering at VUMC was order set driven (i.e., TSH was often included in the order set used) as opposed to past medical history of an isolated abnormal TSH result driving the ordering of TSH during pregnancy at UKMC. After evaluating TSH results using the non-pregnant reference intervals (NPRI) and TSRI, we found patients in both data sets that had a normal TSH using the NPRI, but an abnormal TSH if the TSRI was utilized (11% and 7.4% for VUMC and UKMC, respectively). Follow up to abnormal results was inconsistent at both institutions and often discordant from the latest evidence based practice guidelines.

Conclusion: Screening for thyroid dysfunction differed by clinical practice at the two academic medical centers. Recommendations should be made at each institution to help align clinical practice with international guidelines for pregnancy testing. The implementation of trimester specific reference intervals and evidence based thyroid function testing algorithms could reduce misinterpretation and inappropriate follow up.

A-214

Performance Evaluation of Thyroid Assays on the Atellica IM 1600 Analyzer

D. Waltrick, C. O. W. Sabino, L. B. Avallone, L. Rodrigues, C. M. M. Dias, E. A. Perobelli, G. Campana. *DASA, São Paulo, Brazil*

Background: Given the significant increase in laboratory tests requests for evaluation of thyroid function in recent years, the use of more reliable, specific and accurate assays is essential. These trials allow earlier diagnosis by improving the therapeutic intervention. The present study aimed to demonstrate the analytical performance of the thyroid profile assays for use in the Atellica IM Immunoassay Analyzer, Siemens Healthineers. Studies were conducted in a large laboratory in Brazil and included imprecision verification, linearity and methods comparison. **Methods:** The repeatability (%CVR) and run-to-run variation (% CVWL) studies were performed according to EP15-A3 and the methods comparison according to EP09-A3, for the Thyroid Stimulating Hormone 3-Ultra (TSH), Total Thyroxine (T4), Total Triiodothyronine (T3), Free Triiodothyronine (FT3) and Free Thyroxine (FT4) assays. The linearity study according to EP06-A for the TSH, T4 and T3 assays. For the imprecision study, two or three concentrations were used; each concentration of QC materials was tested in one run per day, with five replicates per run, for five days, resulting in a total of

25 replicates per sample for each assay. Methods comparison studies were performed using at least 40 serum samples that covered the assay range. The number of linearity material levels ranged up to seven, depending on the assay. Each assay were run in triplicate for each sample concentration. **Results:** The imprecision results are in accordance with analytical quality specifications. The % CVR were from 0.962% to 3.006% and %CVWL were from 1.038% to 6.02% for all Atellica IM Analyzer assays. The comparison results of Passing & Bablock methods (R^2) ranged from 0.9825 to 0.9934. In the relative difference graph 98% are within the total error calculated for each difference, according to the specification of analytical performance defined by the laboratory (EDA ≥ 95%). Linearity results were obtained for the TSH, T4 and T3 assays. The assays tested on the Atellica IM Analyzer and the ADVIA Centaur XPT demonstrated excellent agreement. **Siemens Healthineers supported the studies by providing systems, and reagents.*

A-215

Breaking Paradigms - Rationalizing the Post Insulin Cortisol Stimulus Test in Children

Y. Schrank, A. F. D. F. Pedrozo, P. M. C. Araújo, M. M. C. Pinheiro, D. V. Gomes, A. S. Santos, G. A. Campana, M. D. Freire, R. Fontes. *DASA - Brazil, Rio de Janeiro, Brazil*

Background: The insulin tolerance test (ITT) is frequently used for simultaneous evaluation of the hypothalamic-pituitary-adrenal axis and growth hormone secretion in children. In contrast to extensive published literature pertaining to GH response following ITT, only few reports have considered the appropriate duration of the test in the context of evaluation of adrenal insufficiency. We therefore undertook a retrospective review of all ITT carried out in a Laboratory reference unit - Rio de Janeiro - Brazil. **Objective:** To determine timing of the peak cortisol response to ITT and appropriate duration of the test in a children cohort. **Methods:** We performed a retrospective analysis of children and adolescents submitted to insulin tolerance test in a laboratory referral center over a 5-year period (2012 - 2017). Inclusion criteria were age ≤ 18 years, adequate hypoglycemia, defined as a glucose nadir ≤ 2.2 nmol/L (≤ 40 mg/dL) and a normal response of cortisol to the test. A normal response to the test was defined as a peak cortisol (maximum absolute concentration) at any time of the test ≥ 400 nmol/L (14.4 µg/dL). Patients with known or suspected organic hypothalamic-pituitary diseases and patients receiving glucocorticoid medication were excluded. One hundred and twenty-four subjects (86 males) met the criteria. Blood samples were collected at time 0, during hypoglycemia and 30, 60 and 90 minutes after hypoglycemia. Regular insulin was applied as a bolus injection and dose ranged from 0.075 - 0,15 U/kg. Glucose, cortisol and growth hormone were measured in all samples.**Results:** One hundred and twenty-four patients were eligible for inclusion in our study, 69% of which were male. The median (5th and 95th centiles) peak cortisol was, 579 (566-742) nmol/L [21 (20.5-26.9) µg/dL] for the whole group. There was no correlation between glucose nadir and peak cortisol (r=0.09, p=0.3). Peak cortisol response occurred at time 0, during hypoglycemia, 30, 60 and 90 minutes after hypoglycemia in 1 (0.8%), 6 (4.8%), 75 (61.3%), 31 (25%) and 11 (8.9%) subjects, respectively. Two (1.6%) subjects with a peak cortisol response later than 30 minutes after hypoglycemia showed a cortisol response ≥ 400 nmol/L (14.4 µg/dL) only after that point. **Conclusion:** Since hypoglycemia represents the drive for cortisol stimulus, it seems rational to collect samples in accordance with the documentation of the event and not in pre-established times. A limited number of previous studies have explored the possibility of reducing the number of blood samples on cortisol provocative tests in children. Based on our results only two patients would have been missed (1.6 % false positive result) if we would have interrupted the test after blood sampling 30 minutes after hypoglycemia. Therefore, we conclude that ITT can be optimized, without compromising its specificity, by collecting only three samples: basal, during hypoglycemia and 30 minutes after hypoglycemia. Such a procedure would result in a less cumbersome test and in a significant financial saving.

A-216

Assessment of Thyroid Antibodies Profile among Patients Who Measured Antibodies against Cyclic Citrullinated Peptides (aCCP)

P. Araujo[1], A. Perozo[1], Y. Shrank[1], V. Vettori Tessari[2], M. Pinheiro[1], D. Gomes[1], G. Campana[2], L. Carpinetti Vieira[2], M. di Calafiori Freire[1], R. Fontes[1]. *[1]DASA, Rio de Janeiro, Brazil, [2]DASA, São Paulo, Brazil*

Background: Rheumatoid arthritis (RA) is a chronic, autoimmune disease with a prevalence of 0.5% to 1% in the general population. Testing for anti-cyclic citrullinated peptides (aCCP) is a useful and most commonly used tool for the diagnosis of RA (sensitivity of 57% and specificity of 96%). An association between RA and thyroid dysfunction with or without autoimmune origin has been reported in 6% to 34% of patients with RA. Conversely, when presence of thyroid antibodies (TAb) is considered, despite normal thyroid function, the prevalence can rise up to about 38%. Therefore, the aim of this study was to assess the prevalence of TAb among patients who measured aCCP in a private laboratory. **Methods:** Evaluation of anonymously data collected from exams performed in a private laboratory from Rio de Janeiro, Brazil, from July 2017 to June 2018. Among patients who have aCCP measured during this time interval, we evaluated the status of the TAb: anti-thyroid peroxidase (TPO), anti-thyroglobulin (ATG) and anti-thyroid-stimulating-hormone-receptor antibody (TRAb). Statistical analyses were performed using R version 3.5.2. Chi-square test was used for categorical variables and Wilcoxon-Mann-Whitney test to compare medians. Statistical significance was set as p-value <0.05. **Results:** A total of 4395 (81.71% female) people who measured aCCP were evaluated. Mean age of female and male groups were respectively 54.8 (± 15.9) and 54.57 (± 17.3) years. aCCP results were considered undetermined (7.0-10.0 U/mL), negative (<7.0 U/mL) and positive (>10.0 U/mL) while TPO, ATG and TRAb were considered negative and positive according to the reference value (respectively, positive>34 U/mL; positive>115 U/mL and positive>1,75 UI/L). The aCCP undetermined group was too small for statistical evaluation, therefore was disregarded. A total of 2518 patients measured both aCCP and TPO with a TPO positive/aCCP positive versus TPO positive/aCCP negative relationship of 20.07%(n=53/264) versus 15.21%(n=343/2254; p=0.049). aCCP and ATG was measured in a total of 1965 patients with a ATG positive/aCCP positive versus ATG positive/aCCP negative relationship of 18.55%(n=36/194) versus 16.20%(n=287/1771; p=0.461). Results of aCCP and TRAb of 364 patients showed a TRAb positive/aCCP positive versus TRAb positive/aCCP negative relationship of 23.33%(n=7/30) versus 15.21%(n=51/334; p=0.3705). An integrated analysis of the profile of all 3 TAb among 2597 patients who measured aCCP showed a TAb positive/aCCP positive versus TAb positive/aCCP negative relationship of 26.18% (n=72/275) versus 21.61% (n=502/2322; p=0.09949). Median TPO was significantly higher in seropositive than seronegative aCCP patients (11.05 (IQR=15.85) and 9.7 (IQR=9), respectively) (p=0.01037). Similarly, TRAb was significantly higher in seropositive than negative aCCP patients (0.55 (IQR=0.8) and 0.4 (IQR=0.5), respectively) (p=0.03054). ATG was not significantly different between aCCP groups (p=0.06458). **Discussion:** This study showed that there is a significant higher prevalence of TPO positivity among patients who are aCCP positive. No correlation was observed for ATG, TRAb or when all TAb were analyzed together. Our findings are similar to previous data, suggesting that measurement of serum TPO may be beneficial for RA patients, especially for the ones who have thyroid disease family history or slightly altered TSH, or even when autoimmune poliglandular syndrome is suspected.

A-217

Evaluation of ARKRAY ADAMS HA-8180V for HbA1c Measurement

G. Altawallbeh, V. Makky, A. K. Saenger, J. Peters, A. A. Killeen. *University of Minnesota, Minneapolis, MN*

Objective: We evaluated ADAMS™ HA-8180V, a fully automated ion-exchange HPLC system, for routine hemoglobin A1c measurement in comparison with Tosoh G8 analyzer for normal hemoglobin and with the Trinity analyzer for variant hemoglobins. **Methods:** The analytical performance (linearity, precision, carryover, and sample stability) was assessed based on the Clinical and Laboratory Standards Institute (CLSI) and manufacturer guidelines. Two levels of a commercially available control and Bio Rad Linearity Kit were tested. A comparison of the HA-8180V against the Tosoh G8 was performed for 100 whole blood samples. HA-8180 Variant mode was also compared against the Trinity A1c analyzer for 50 samples containing hemoglobin variants (HbC 14, HbS 14, HbD 12, HbE 10). **Results:** Inter-assay and intra-assay CVs are summarized (Table). Linearity: $r^2 = 0.9998$ in the concentration range 3.4−18.4 % HbA1c, carry-over: 0.0%, and four freeze-thaw cycles stability were excellent. Method comparison of ADAMS™ HA-8180 Variant mode and Tosoh G8 demonstrated a high concordance between both measurement procedures for 100 whole blood samples. Passing-Bablok regression showed a slope of 1.000 (CI: 1.000 to 1.000), and intercept of -0.100 (CI: -0.100 to -0.100) for HbA1c and yielded very high correlation coefficient (r > 0.995). Bland-Altman plot showed a mean difference of -0.096%. The correlation coefficient, r, between the HA-8180V and the Trinity A1c analyzer for HbA1c variants was > 0.973. Passing-Bablok regression showed a slope of 0.976 (CI: 0.917 to 1.036), and intercept of 0.130 (CI: -0.282 to 0.567) for A1c variants. The mean of difference was -0.082%. **Conclusion:** ADAMS HA-8180V A1c analyzer demonstrated high analytical performance compared to the Tosoh G8 analyzer. Inter and intra-assay CVs are both lower than the CVs obtained by the reference method. The HA-8180V shows good correlation with the Trinity for hemoglobin variants.

	HA-8180V		Tosoh G8	
HbA1c	**Mean**	**CV (%)**	**Mean**	**CV (%)**
	Inter-assay Precision			
Low	5.1%	0.75%	5.1%	1.07%
High	8.0%	0.63%	8.1%	0.69%
	Intra-assay Precision			
Low	4.9%	<0.10%	4.8%	0.90%
High	9.6%	0.50%	9.7%	0.05%

A-218

Comparison of Five Bioavailable Testosterone Testing Methods

C. Hamilton, K. Robyak, P. McGhee, C. Pederson, M. Straub, S. Scott, T. Neibauer, W. Salter, M. Creer, Y. Zhu. *Penn State University College of Medicine, Hershey, PA*

Introduction: Bioavailable Testosterone (BT) includes free and albumin-bound testosterone. BT is the recommended second-level test for suspected increases or decreases in physiologically active testosterone. BT results can be obtained by several different means using serum or plasma. One common approach is to assay Total Testosterone (TT) and Sex Hormone Binding Globulin (SHBG) and utilize an algorithm that enables the calculation of Free Testosterone (cFT) as well as Bioavailable Testosterone (cBT). Another method employs the use of a 50% Ammonium Sulfate Precipitation (NH_4SO_4 PPT) assay to more directly measure the BT fraction. The objective of this study was to assess the accuracy and variability of each method/algorithm to determine the optimal BT assay to offer clinicians in our institution.

Methods: Forty-nine serum specimens with known cBT values (1.2 to 441.7 ng/dl) were obtained from a large national reference laboratory. The reference lab used a proprietary, modified Vermeulen algorithm along with a TT by LC-MS/MS assay and an SHBG Electrochemiluminescence immunoassay (ECLIA) to calculate the cBT. The same specimens were also tested in our laboratory using an LC-MS/MS TT assay and an SHBG ECLIA. The in-house TT and SHBG results were then used in four algorithms (Vermeulen, Sodergard, Emadi-Konjin, and Morris) of interest to calculate final in-house cBT results. A 50% Ammonium Sulfate Precipitation assay was also run to produce BT results. Deming regression analysis (EP Evaluator, Data Innovations) was used for method comparison and Microsoft Excel was employed for the calculation of means and standard deviations (SD) of percent bias.

Results: Method comparison statistics were generated for each in-house method (y) compared to the reference lab (x). Vermeulen (y_1): y_1 = 0.849x - 2.0 (R = 0.998, SEE = 7.63 ng/dl), mean bias = -13.0% ± 6.3% (mean ± SD) ; Sodergard (y_2): y_2 = 1.078x - 6.0 (R = 0.996, SEE = 15.46 ng/dl), mean bias = 13.0% ± 14.4%; Emadi-Konjin (y_3): y_3 = 0.430x - 6.29 (R = 0.973, SEE = 15.71 ng/dl), mean bias = -68.5% ± 8.0%; Morris (y_4): y_4 = 0.540x - 4.02 (R = 0.969, SEE = 21.17 ng/dl), mean bias = -23.3% ± 28.8%; NH_4SO_4 PPT (y_5): y_5 = 0.883x - 10.93 (R = 0.988, SEE = 21.85 ng/dl), mean bias = -28.9% ± 12.6%. Compared to the reference lab cBT, only the Vermeulen and Sodergard algorithms had correlation coefficients >0.99. The same two methods were shown to be the most accurate as well described by a -13.0% bias for the Vermeulen and a 13.0% bias for the Sodergard methods. Finally, the least variable method was again the Vermeulen method with a 6.3% bias SD.

Conclusions: Compared to the reference lab results, the Vermeulen algorithm is the most accurate and least variable method in our laboratory. The statistical analysis supports the use of the Vermeulen algorithm along with the in-house Total Testosterone LC-MS/MS and SHBG ECLIA assays to produce high quality cBT results that agree very favorably with a reputable national reference lab.

A-219

Role of the Body Mass Index on Determination of TSH Reference Interval in a Euthyroid Population

M. C. G. CASTELO[1], C. M. M. Ponte[2], R. M. Montenegro Júnior[2], L. L. A. Cavalcante[1], S. P. Bandeira[1], F. L. B. Furtado[2], I. V. Rocha[1], G. A. Campana[3]. *[1]DASA, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]DASA, São Paulo, Brazil*

Background: Formally, body composition has not been considered as a relevant factor for determination of TSH reference interval (RI). However, bidirectional association between thyroid status and body mass index (BMI) has been described, even in individuals with normal thyroid function. The objective of this study was to evaluate the impact of BMI on the selection of the reference individuals for the determination of TSH RI in a euthyroid population. **Methods:** A cross-sectional study was conducted with 272 euthyroid individuals aged between 18 and 50 years old. The subjects were assessed by medical interview, anthropometric and laboratory evaluation. TSH, total T3, free T4, antithyroid peroxidase antibody (TPOAb), and thyroglobulin antibody (TgAb) were determined by electrochemiluminescence - Modular Analytics E170-Roche equipment. The TSH RI was calculated in euthyroid individual group (reference individuals; n=272) and in a subgroup of euthyroid individuals with BMI < 25 kg/m2(normal BMI; n=129). The 2.5th and 97.5th percentiles of the distribution curve correspond to lower and upper TSH levels. The 95% confidence interval (95%CI) was calculated for lower and upper TSH limits. **Results:** In the reference individual group, the mean age was 34.5 ± 11.2 years and 67.2% (183) were female. TSH mean concentration was 1.74 ± 0.96 mIU/mL and TSH RI was 0.56 to 4.40 mIU/mL (95%CI = 2.5th: 0.49-0.62; 97.5th: 3.94-4.89 mUI/mL). Prevalence of overweight was 37.5% (102) and obesity was 16.5% (45). TSH mean in individuals with BMI < 25 kg/m^2 was 1.74±0.96 mIU/mL and in IMC ≥ 25 kg/m^2 was 1.71±0.90 mIU/mL (p= 0.770). In the subgroup normal BMI (n=129), the mean age was 31.5 ± 10.5 years and 70.5% (91) were female. The TSH RI in this subgroup was 0.59 to 4.43 mIU/mL (95%CI = 2.5th: 0.54-0.68; 97.5th: 4.00-5.65 mUI/mL). **Conclusions:** In this study, the presence of overweight or obesity had no influence on the determination of TSH reference interval in a sample of euthyroid individuals. Considering the increasing prevalence of overweight and obesity in the world, studies with a larger number of individuals may be necessary to confirm these findings.

A-220

Development of a Liquid-Stable HbA1c Calibration Verification Control Kit to Characterize Method Linearity and Validate the Reportable Range

D. Avery, J. Pawlak, M. Sweatt, R. Ito. *LGC Maine Standards, Cumberland Foreside, ME*

Introduction: HbA1c is a form of hemoglobin where one or both N-terminal valines of the β-chain are glycated. The glycosylation process is non-enzymatic, irreversible and continually occurs throughout the lifespan of the red blood cells (8 to 12 weeks). This makes HbA1c an excellent biomarker to monitor glycemic control and therapeutic interventions for diabetic management.

HbA1c assays have been cleared by the FDA as non-waived laboratory tests and therefore, in accordance with CLIA '88, clinical laboratories are required to verify the manufacturer's assay performance characteristics, including accuracy, precision and reportable range (AMR).

LGC's objective was to develop a five-level, liquid-stable, human whole blood based HbA1c control kit to meet the calibration verification and linearity needs of clinical laboratories supporting HPLC, antibody (immunoassay) and enzymatic based methods.

Methods: Two VALIDATE® HbA1c formulations were developed: 605 and 605to. Each was derived from human whole blood and formulated according to CLSI EP06-A into five equal-delta concentrations to cover the manufacturer's reportable ranges for HbA1c: (1) 605 - Roche's cobas® HB-W3 A1-W3 assay - 4.2 - 20.1% and (2) 605to - Tosoh Automated Glycohemoglobin Analyzer HLC-723G8 % hemoglobin A1c (HPLC): 3.4 - 18.8%.

For each level, samples were tested either in triplicate on the Roche cobas® 6000 analyzer (605) or in duplicate on the Tosoh G8 analyzer (605to). Recoveries were evaluated for mean, SD, and linearity using MSDRx®, LGC's proprietary linearity software. Limits were applied as 50% of the total allowable error (TE_a) for HbA1c: 0.25% or 6%, whichever is greater. To establish product stability claims, samples were subjected to one freeze-thaw cycle, followed by a 72 hour, 30°C stress condition and four subsequent freeze-thaw events.

Results: For both formulations, all levels were stable, recovering within ±10% of the recovered values on the date of manufacture in both stress and real-time stability studies. Mean recoveries were linear and within the applied TEa limits through the manufacturers' reportable ranges. Further, the 605to formulation limited aberrant peaks caused by hemoglobin degradation and produced acceptable chromatograms. For 605 and 605to, stability was established at 15 months, including four freeze-thaw events.

Conclusion: VALIDATE® HbA1c, as five level, liquid-stable, ready-to-use control, are fit-for-purpose as calibration verification materials. The 605 formulation preserves the specific epitope recognized by the antibodies for HbA1c and the 605to formulation preserves hemoglobin integrity, optimizing it for HPLC assays. Formulations provide coverage of the respective manufacturer's claimed reportable range. The products are listed with the FDA and are commercially available.

A-221

Practices and Recommendations for Reporting Quantitative Human Chorionic Gonadotropin (hCG) Test Results

R. R. Crosbie[1], D. G. Grenache[2]. *[1]Department of Pathology, University of New Mexico, Albuquerque, NM, [2]TriCore Reference Laboratories, Albuquerque, NM*

Background: Quantitative serum human chorionic gonadotropin (hCG) tests are commonly used to determine a woman's pregnancy status. Test results are discrete and interpretation is usually accomplished by comparison of the numerical result to a reference interval or cutoff. Despite widespread availability of quantitative hCG testing, no clear standards or best-practice recommendations exist to guide clinical laboratories in establishing these thresholds.

Methods: A voluntary, seven-question, supplemental questionnaire was included in the College of American Pathology (CAP) general chemistry C-B 2017 survey. These questions were designed to determine the different hCG reference intervals that clinical laboratories use to report results of quantitative hCG tests and to determine the hCG cutoffs used to assign interpretive comments.

Results: Of the 6,433 laboratories surveyed, complete or partial survey responses were received from 3,568 (55%). 31% of laboratories provided a single cutoff when reporting quantitative hCG test results with 22% using a cutoff of less than 5 IU/L and 42% using a cutoff of 5 IU/L to identify a positive result. 20% use a cutoff that falls between 5 and 25 IU/L, and 14% used a cutoff of 25 IU/L. 53% of laboratories provided interpretation of quantitative hCG results. Of these, 33% reported negative and positive interpretations, 30% reported only negative interpretations, 20% reported negative, indeterminate, and positive interpretations and 13% reported only positive interpretations. Collectively, among laboratories that provide interpretations, 85% included a negative interpretation compared to 67% for positive and 24% for indeterminate interpretations. Laboratories without indeterminate interpretations most frequently used a cutoff of greater than 5 IU/L (46%) to discriminate positive and negative results. In contrast, laboratories that reported all three interpretations most frequently used a cutoff of less than 5 IU/L (65%) to identify negative results, greater than 25 IU/L (71%) to identify positive results, and interpreted results as indeterminate if the value was between 5 IU/L and 25 IU/L (52%). 60% of respondents reported using gestational age-based hCG reference intervals. Only 9% of respondents indicated the use of age-based hCG reference intervals for non-pregnant females. Respondents most often used hCG reference intervals stated in product inserts (48%) followed by scientific literature (8%) and internal laboratory studies (5%). 15% reported using a combination of more than one source. 25% of laboratories were not certain of their hCG reference interval source.

Conclusion: These data demonstrate wide variation in the reporting of quantitative hCG results and highlights the need to develop best-practice guidance on the topic. Despite a well-established, validated hCG reference limit of less than 5 IU/L for non-pregnant women, only 34% of reporting laboratories use this cutoff. Studies have also suggested that age-based hCG reference intervals would be clinically useful, however only 9% of laboratories report doing so. In contrast, 60% of laboratories use gestational age-based hCG reference intervals despite their limited clinical usefulness. Defined, data-driven guidelines for reporting quantitative hCG test results could deliver more consistent result interpretation.

A-222

Does Reverse T3 Physiologically Rise with Age?

R. Fontes, P. M. C. Araujo, D. V. Gomes, M. M. C. Pinheiro, A. S. Santos, A. F. D. F. Pedrozo, M. d. Freire, G. A. Campana, Y. Schrank. *DASA - SA, Rio de Janeiro, Brazil*

Background: Thyroid stimulating hormone (TSH) shows a progressive increase with age in healthy subjects and this is associated with reduced mortality. Some studies have reported an increase in Reverse T3 (RT3) with age, but there are a few studies with subjects without debilitating diseases, so that it is questioned whether this finding are present in all the elderly or if they are characteristic of those with poor health status. **Objectives:** To evaluate RT3 levels in the elderly without debilitating diseases; to compare RT3 levels with those of young people; and to evaluate if there is correlation of RT3 with TSH and thyroid hormones (TH). **Patients and Methods:** 249 subjects were studied, 187 (75%) females, segmented by age: young people (under 60), elderly (60 to 79 years), and very old (over 80 years). We selected patients without past/present, or family history of thyroid disease, previous thyroid surgery, goiter, smoking habit, use of possible interfering drugs in TSH or TH, antidepressants, hospitalization in the past six months, and pregnant females, with negative thyroid anti-thyroperoxidase (TPOAb), and with TSH within the reference interval (RI) according to age: Under 60 years: 0.4-4.3 mUI/L, 60 to 79 years:0.4-5.8 mUI/L, and over 80 years:0.4-6.7 mUI/L. T3R RI was 13 to 50,2 ng/dL. Total T3 (TT3), free T3 (FT3), and free T4 (FT4) were also analyzed. The significance level was 0.05. **Results:** TH had normal distribution, and the results are presented in means and standard deviations. TSH had non-normal distribution and the results are presented in median and 25% and 95% percentiles. RT3 increased with age, remaining within the reference interval (RI) in all age groups (ng/dL): Under 60 years: 18,62 ± 5,46; 60 to 79 years: 23,87 ± 5,65; 80 years and over: 27,32 ± 8,81. Applying ANOVA test, f= 13.67995, $p < 0.00001$. RT3 was lower in 20 to 59 years compared to 60 years and over: t=4.3403, p=0.000024. RT3 showed a positive correlation with TSH: R=0.1467/R2= 0.0215, and negative correlation with FT4, TT3 and FT3 (R=- 0.4781/R2=0.2286, R=- 0.399/R2=0.0001, and R=-0.3551/R^2=0.1261, respectively. **Discussion:** In this group of elderly people, without debilitating conditions, RT3 increased with age remaining within the reference values, accompanying the elevation of TSH, and in the opposite direction to that of the TH. TSH increase is a physiological event with age, and a low activity of TH might be beneficial in the elderly. It is assumed that the lower TH and the high RT3 could serve as an adaptive mechanism to prevent catabolism in the elderly. This could be explained because of the decrease in the deiodinase type 1 (D1) activity leading to a decrease in T3, with reduction of the peripheral conversion of T4-T3, and an increase of RT3. An additional factor to increase RT3 could be the decrease of its uptake and degradation in the liver, due to the aging process itself.

Conclusion: Elevation of RT3 with age, within the limits of the reference range, appears to be a physiological event in parallel with the elevation of TSH.

A-223

Applicability of Laboratory Database as a Tool for Screening Homozygous Familial Hypercholesterolemia (HoFH)

L. L. A. CAVALCANTE[1], M. G. CASTELO[1], F. L. B. FURTADO[2], S. P. BANDEIRA[1], T. A. C. PALHANO[1], C. S. CÂNDIDO[1], W. T. F. LUCENA[1], E. C. LUNA NETA[1], H. F. ALVES[1], G. A. CAMPANA[3]. *[1]DASA, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]DASA, SÃO PAULO, Brazil*

Background: Familial hypercholesterolemia (FH) is an autosomal dominant hereditary disease characterized by high serum levels of low-density lipoprotein (LDL-C) and total cholesterol. It presents a high risk of early cardiovascular disease in both genders. The homozygous form is rare, occurs in about 1: 1,000,000 individuals and is characterized by elevated LDL-C levels as high as 500 mg/dL (12.95mmol/L). **Objective:** To evaluate the applicability of the Diagnostics of America (DASA) laboratory database as a tool for screening individuals with HoFH form. **Method:** A cross-sectional study was conducted to screen LDL-C serum above 500 mg/dL (12.95 mmol/L) in population from DASA laboratory database (January 2015 to march 2018). The LDL value of each sample was calculated by the Friedewald formula. Patients hospitalized or with renal failure were excluded from the analysis. **Results:** Fifty six individuals with LDL-C above 500mg /dL (12.95 mmol/L) were detected, which corresponds to a prevalence of 17: 1,000,000 exams. There was a male predominance (51.8%) in the group evaluated. The mean serum levels of LDL-C were 622 ± 130mg / dl (16.10 ± 3.3mmol/L). **Conclusion:** This tool seems to present high sensitivity for screening high levels of LDL-C, which is one of the defining elements of true cases of HoFH. The laboratory database can optimize diagnosis of rare diseases.

A-224

Determination of Insulin Reference Intervals for Adolescents from 10 to 19 Years Old in Brazil, Using an Indirect Methodology

M. C. Freire[1], I. Bouzas[2], T. S. P. Souza[1], P. R. Telles-Dias[3]. *[1]DASA, RIO DE JANEIRO, Brazil, [2]State University of Rio de Janeiro, RIO DE JANEIRO, Brazil, [3]Fluminense Federal University; State University of Rio de Janeiro, RIO DE JANEIRO, Brazil*

Background: The determination of reference intervals (RI) for clinical tests is fundamental for the correct diagnosis and follow-up of health-related issues, and on them rely 70% of medical decisions. However, conventional determination of RI is costly and time-consuming, involving sampling and an effort to select healthy participants. As a consequence, it is not uncommon that laboratories use RI available from test kits manufacturers, or validation of RI determined in a different population, without taking into account possible differences related to gender, age, ethnicity, cultural and socioeconomic aspects. Despite recommendations of CLSI, that ideally laboratories should determine their own RI, specific for the population they assist, only around 25% of laboratories do it for vulnerable populations, as adolescents. A more economical alternative to the direct determination of RI is the indirect method, through a retrospective analysis of data stored in Laboratory information system databases. In this study, we intend to use this last methodology to help determine IR of insulin among 10 to 19 years old adolescents - as regional insulin RI standards were not determined yet. **Methods:** To determine the RI, mathematical algorithms will be used to separate laboratory results samples from individuals considered “healthier”. For the statistical model, we used all insulin results for the studied age group stored during the period of one year. After data cleaning, algorithms were then used to fit the data. The statistical model for the estimation of RI used a variety of additional information from the data base, especially results of other analites that were performed at the time insulin was measured, - and presented significant correlation ($p <.001$) with insulin. Records in which correlated analites presented abnormal were excluded from the final file used to determine RI. Taking it into account, the statistical distribution of the individuals considered “healthier” was determined. Other inclusion/exclusion criteria used, besides abnormal values of correlated variables were: outliers; repetition of insulin exams; use of prescription medicines. After obtaining the distribution parameters, the IR is then estimated. **Results:** Up to now, it was carried out a bibliographical review on the mathematical methods used; cleaning and preparation of the data for analysis; the study of clinical aspects related to insulin in the age group of interest (specially those related to normal levels specific to this age group), the study was submitted and received ethical approval from the State University of Rio de Janeiro’s review board. We performed pilot calculations on the sample initially planned (12 months interval) and decided to extend to a wider observational interval (4 years), and include additional analites in the algorithm. Pilot study was performed with 18,583 records (14,075 of which were used after initial cleaning). Correlated variables were: BMI, glucose, total cholesterol, HDL, age, TG, PTH, ALT, Somatostatin, etc. These variables will be re-assessed for correlation on the new 4 years interval file, together with the new variables included. **Conclusion:** The parameters we aim to evaluate in this study, can represent new decision limits for the Brazilian population, contributing to improve diagnosis in our country.

A-225

Effect of Acute Aerobic Exercise on Arterial Stiffness and Thyroid Hormone in Subclinical Hypothyroidism

M. Masaki, K. Koide, A. Miyazaki, M. Koshiba. *Hyogo College of Medicine, Nishinomiya, Japan*

Background: Acute exercise has been reported to increase thyroid hormone levels and decrease arterial stiffness in healthy young subjects. However, the effect of acute aerobic exercise on circulating thyroid hormone levels and arterial stiffness in patients with subclinical hypothyroidism remains unclear. The aim of this study was to investigate the effects of acute aerobic exercise on arterial stiffness and thyroid hormone levels, and any relationship between these endpoints, in patients with subclinical hypothyroidism. **Methods:** We studied patients with untreated subclinical hypothyroidism ($n = 53$, 65 ± 12 years old) compared with euthyroid subjects ($n = 55$, 64 ± 10 years old). Exercise analysis was performed with a ramp cycle ergometer test. Arterial stiffness (cardio-ankle vascular index, CAVI) was measured at baseline and 5 minutes after exercise. We collected participant blood samples for serum thyroid-stimulating hormone (TSH) and free thyroxine (FT4) measurements before and 5 minutes after exercise. **Results:** The CAVI and serum TSH levels significantly decreased after exercise in the subclinical hypothyroidism group (CAVI; 8.1 ± 1.6 vs. 8.5 ± 1.5, $p < 0.001$, TSH; 6.7 ± 1.4 vs. 7.6 ± 1.2 µIU/ml, $p < 0.001$) and euthyroid group (CAVI; 7.6 ± 1.0 vs. 8.3 ± 0.9, $p < 0.001$, TSH; 2.2 ± 1.1 vs. 2.4 ± 1.2 µIU/ml, $p = 0.005$). The changes in CAVI from baseline compared with after exercise were lower, in absolute values, in the subclinical hypothyroidism group than in the euthyroid group (subclinical hypothyroidism group vs euthyroid group; ΔCAVI: -0.4 ± 0.6 vs. -0.7 ± 0.7, $p = 0.012$). The changes in serum TSH from baseline to after exercise were higher, in absolute values, in the subclinical hypothyroidism group than in the euthyroid group (subclinical hypothyroidism group vs euthyroid group; Δ serum TSH: -1.3 ± 1.4 vs. -0.3 ± 0.5, $p < 0.001$). The changes in CAVI from baseline to after exercise were negatively correlated with changes in TSH ($r = -0.32$, $p = 0.038$) in the subclinical hypothyroidism group. In conclusion, acute aerobic exercise decreased both arterial stiffness and serum TSH levels in patients with subclinical hypothyroidism and euthyroid subjects. While the absolute change in arterial stiffness decreased, the absolute change in serum TSH levels increased in patients with subclinical hypothyroidism compared with euthyroid subjects. **Conclusion:** These data suggest that subclinical hypothyroidism reduces CAVI during acute aerobic exercise. Further changes in absolute levels of serum TSH in subclinical hypothyroidism may result in reduced CAVI improvement by acute aerobic exercise.

Tuesday, August 6, 2019

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

Factors Affecting Test Results

A-226

Effect of Sex, Age and Season on 25 Hydroxyvitamin D: A Data Mining Study

D. Wang[1], S. Yu[1], Y. Yin[1], H. Li[1], X. Cheng[1], t. Xu[2], x. guo[1], r. zhang[1], x. Gao[1], L. Qiu[1], y. zou[1]. [1]*Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China,* [2]*Department of Epidemiology and Biostatistics, Institute of Basic Medical Sciences, Chinese Academy of Medical Science & School of Basic Medicine, Peking Union Medical College Beijing 100005, China, Beijing, China*

Background: 25-Hydroxyvitamin D [25(OH)D], which plays a vital role in bone and mineral metabolism, is also associated with diabetes, cancer, and other diseases and is considered the most accurate biomarker of serum vitamin D levels. This study aimed to explore the effect of sex, age, and season on 25(OH)D, using clinical big data, in order to determine which factor has the greatest impact on 25(OH)D measurements.

Methods: Clinical big data was collected from the Laboratory Information System at the Peking Union Medical College Hospital between December 2014 and October 2018. A total of 30642 25(OH)D measurements were obtained and analyzed. 25(OH)D concentrations were measured using a Roche e601 automatic analyzer. Linear regression was used to explore the effect of sex, age, and season on 25(OH)D level.

Results: The median serum 25(OH)D concentration was significantly higher in males than in females ($p < 0.001$). The median serum 25(OH)D concentration at the peak level in August was 22.1 ng/ml. Linear regression revealed that summer had the highest impact on 25(OH)D (standardization coefficient = 0.273). Further, the serum 25(OH)D level in males was higher than in females. There was no significant difference in 25(OH)D levels between age groups.

Conclusion: Sex and season significantly affected serum 25(OH)D levels, with the most significant impact being observed in the summer season; levels showed a substantial seasonal variation with a nadir in early spring and peak at the end of the summer.

A-227

Parameters for Validating a Hospital Pneumatic Tube System: Lessons Learned from Closing a Satellite Laboratory

C. W. Farnsworth, D. M. Webber, J. A. Krekeler, M. M. Budelier, N. L. Bartlett, A. M. Gronowski. *Washington University, St. Louis, Saint Louis, MO*

Background: Pneumatic tube systems (PTS) provide rapid transport of patient blood samples, but the physical stress of PTS transport can damage blood cells and alter test results. False elevations in LDH, K+, and HI have been associated with prolonged time in the PTS and excessive number of accelerations with g-forces >3. Despite the known impact of PTS transport on cellular lysis, there is no consensus on how to best evaluate a PTS for its effects on clinical test results.

Methods: We compared two accelerometers and evaluated multiple PTS routes. Within and between route variabilities in PTS forces were assessed. Response curves were generated that demonstrate the relationship between the number and magnitude of PTS accelerations on plasma lactate dehydrogenase (LDH), hemolysis index (HI), and potassium (K+) in blood from volunteers. Extrapolation from these relationships were used to predict PTS routes that may be prone to false laboratory results. Historical data and prospective patient studies were compared to predicted effects.

Results: The maximum recorded g-force was 10 g for the smartphone and 22 g for the data logger. Given the limitations of the smartphone at higher g-forces, the data logger was used for all further experiments. There was considerable day-to-day variation in the magnitude of accelerations (CV 4-39%) within a single route and between eight different routes. We found that males had greater susceptibility to PTS-induced increases in LDH and HI than females and there was an inverse relationship between the number of blood tubes transported in a carrier and the magnitude of g-forces. The routes that experienced accelerations with the greatest g-forces were Route 5 (a cardiac floor) (mean # accelerations >15 g = 44, 95% CI; 35-52) and Route 8 (an outpatient cancer center) (mean # accelerations >15 g = 29, 95% CI; 22-35). Response curves were generated based on parameters collected from the data logger. The linear relationship between

LDH and accelerations within the PTS revealed two PTS routes predicted to cause clinically significant increases in LDH (defined as >20%); Route 5 (27.2±6.3%) and Route 8 (23.4±6.3%). PTS Route 5 also had the largest predicted impact on HI (31±7.2) and K (0.11±0.02 mmol/L). An astute physician in our out-patient leukemia/lymphoma cancer center perceived an increase in the number of samples with falsely elevated LDH after closing a satellite laboratory that was located within the same building as the cancer center. The new route from the cancer center to the core laboratory was Route 8. The predicted increase in LDH was similar to that observed in patient results after closing the satellite laboratory.

Conclusions: Hospital PTSs can be validated using a data logger with a 3-axis accelerometer and blood samples from healthy male subjects can be used to generate response curves to estimate increases in LDH, HI, and K+. Implementation of this method for validation is relatively inexpensive, simple, and robust. Importantly, validation of the PTS allows for modifications that limit pre-analytic variability and ensures accurate reporting of patient results.

A-228

Change from Beckman Coulter DxC to AU Clinical Chemistry Systems - Effect on Hemolysis Index Measurements

R. Hawkins. *Tan Tock Seng Hospital, Singapore, Singapore*

Background: Sample hemolysis assessment by automated spectrophotometry is a routine procedure in many clinical laboratories, allowing easy assessment of hemolysis rates by location and providing data for sample quality improvement monitoring. We describe the effect on our reported sample hemolysis rates by changing from the Beckman Coulter DxC-800 to the AU-5800 as our analytical platform.

Methods: From 2005 to mid 2018, our main chemistry analytical system was a Beckman Coulter Power Processor system with 3 DxC-800 clinical chemistry analysers. We routinely performed hemolysis index measurement on all specimens and used the results to filter analytical results as well as for phlebotomy quality improvement initiatives. In May 2018 2018 we changed to a Beckman Coulter Power Express, with 2 AU-5800 clinical chemistry analysers. Hemolysis measurement was performed on 260 random samples using the DxC and AU systems. The effect of the analyser change on reported hemolysis rates was assessed by extracting hemolysis index results from the laboratory information system for 2017 and 2018.

Results: For the 260 samples directly compared, hemolysis (HE >=1) rates with DXC were much higher (35.7%) than for the AU system (9.6%) with kappa = 0.25. This was reflected in the hospital wide hemolysis rates for July and Aug: 2017 18.0%, 17.6%, 2018: 3.2%, 3.35 and was seen across all sites - the range of monthly hemolysis rates for Jan-April 2018 vs June-Sept 2018 were: ED ICU 26.6-33.8% vs 9.1-10.45; ICU 17.3-19.3% vs 1.2-4.0%; Inpatients 20.2-22.8% vs 2.4-3.7%; Outpatients 7.6-9.8% vs 0-2.3%.

Conclusion: According to Beckman Coulter, the DxC-800 will report a hemolysis index of 1-2 at 0-75 mg/dL hemoglobin while the AU-5800 will report hemolysis as absent at < 50 mg/dL and 1+ at 50-99 mg/dL. By changing to an analyser with a higher cut-off for hemolysis detection, our perceived hemolysis rates have automatically dropped. Laboratories should be aware of inter-instrument differences in serum index cut-offs, even in instruments from the same manufacturer.

A-229

A Case of Falsely Elevated Troponin Levels Using AccuTnI Assay: The Presence of an Unknown Interferant

C. N. Sharp[1], P. Mulunhgwi[1], M. Blank[2], L. Bruck[3], S. A. Jortani[1]. [1]*University of Louisville, Louisville, KY,* [2]*University of Louisville Hospital, Louisville, KY,* [3]*Baptist Health, Louisville, KY*

Background: Falsely elevated troponin levels can result from interferants including heterophile antibodies (HAMA), and endogenous blood components. The true rate of falsely elevated troponin levels is unknown due to many assays being available; a high rate has been reported with Beckman-Coulter's AccuTnI+3 assay. Interference with alkaline phosphatase conjugate in immunoassays has been suggested as another source of falsely elevated troponin levels. We report a falsely elevated cardiac troponin I (cTnI) in a patient with a history of left branch bundle block, who presented to the emergency department (ED) with a fractured femur. The patient was transferred from another hospital after an elevated cTnI value using a Beckman assay, but cTnI

levels with our point-of-care (POC) indicated no elevation in cTnI. This caused us to further investigate the possibility of a falsely elevated cTnI.

Objective: Our objective was to determine the presence and characteristics of the interferant causing falsely elevated cTnI levels.

Methods: Patient samples with elevated cTnI in our lab (AccuTnI, Beckman-Coulter) were re-evaluated using POC (i-STAT, Abbott) and the Architect analyzer (STAT, Abbott). Troponin T was assessed (Troponin T STAT, Roche). Spike-in experiments with the patient's sample using either plasma with elevated cTnI or high-level QC material were analyzed via AccuTnI and STAT assay. To determine if the interferant could be removed, a sample was ultracentrifuged, using a 30 kDa cutoff filter (Centrifree, Millipore). CK-MB was measured using the Beckman Access assay (alkaline phosphatase-based immunoassay), and a Roche Cobas analyzer (non-alkaline phosphatase method).

Results: Patient results obtained from iSTAT and STAT indicated non-elevated cTnI (iSTAT: 0.01, 0.00, and 0.00 ng/ml; STAT: <0.010, <0.010, and <0.010 ng/ml), while cTnI was elevated using AccuTnI (2.95, 2.83, and 1.98 ng/ml). Troponin T was not elevated (0.011, 0.010, and 0.010 ng/ml). Spike-in of patient sample into serum with elevated cTnI or QC material showed a dilutional effect with STAT assay, but not with AccuTnI. Ultracentrifugation indicated that cTnI (26 kDa in size) could not be detected in the ultrafiltrate, suggesting the possibility of a HAMA (retentate: 2.89 ng/ml). Serum was not available for HAMA confirmation. We found that the patient's CK-MB was elevated using the Access assay (5.0 ng/ml; reference range (RR): 0.3-4.0 ng/ml), but within reference range when tested on Cobas analyzer (4.96 ng/ml; RR: 0-5.30 ng/ml).

Conclusion: An interferant in our patient's plasma caused a falsely elevated cTnI and CK-MB on the Beckman analyzer. However, the POC and STAT measurements indicated no elevation of cTnI. This suggests a HAMA is recognizing epitopes on the mouse detection and mouse capture antibodies used in the AccuTnI alkaline phosphatase-based assay, bringing them in close proximity in the absence of antigen, and resulting in a false elevation. Because we do not see this same effect with the alkaline phosphatase-based POC assay (mouse primary and goat conjugate) nor the STAT assay (mouse primary and mouse conjugate antibodies, but an acridinium based detection rather than alkaline phosphatase), we conclude that the HAMA may be specific for the mouse alkaline phosphatase conjugate used in the AccuTnI assay.

A-230

The Pre-analytical Stability of 25-Hydroxyvitamin D: Storage and Mixing

B. Alghamdi[1], R. Alahamdi[1], H. Khalil[1], N. Mohammed[1], S. Bahijri[2], A. Borai[1]. [1]*King Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, Jeddah, Saudi Arabia,* [2]*Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia*

Background: The constantly increasing demand on 25-hydroxyvitamin D (25OHD) testing globally has prompted many laboratories to consider the use of automated immunoassays which are more prone to non-specific interference. Some of these interfaces are thought to be associated with the pre-analytical stability of biological samples. In this study, we have investigated both, storage stability and the effect of mixing blood samples on 25OHD3 levels.

Methods: Blood samples were extracted from 31 healthy donors in plain vacutainer tubes. After extraction, serum samples were stored at -20 C° and analysis was carried out with and without mixing (voretexting) at different time intervals of days (0, 1, 2, 3, 4, 5, 15, and 30). All samples were analyzed using chemiluminescent immunoassay.

Results: Comparing between mean concentrations of subsequent days to day 0 had a significant time effect ($p < 0.05$) except in day 1 ($p = 0.69$) in non-vortexed samples, and day 2, 5 and 30 in vortexed samples. Comparing between vortexed and non-vortexed samples of 25OHD3 of the same day was significant ($p < 0.05$) in day 1, 4, 5 and 30. However, the maximum change value for 25OHD3 after sample storage or mixing (8.9%) is less than the clinically significant reference change value (18%).

Conclusion: 25OHD3 is considered to be stable after long-term of storage and can be analyzed without sample mixing. Such criteria are highly beneficial for both research and diagnostic laboratories.

A-231

Chemistry and Immunoassay Tests Evaluation Using Different Types of BD Vacutainer Barricor Tubes

A. Borai, Y. Abubaker, J. Magjacot. *King Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, Jeddah, Saudi Arabia*

Background: There are different pre-analytical factors affect the reliability of laboratory results. One of those factors is the type of blood collection tube. BD Barricor plasma collection tubes with a mechanical separator have been released recently. Our aim was to evaluate the comparability of routine chemistry and immunoassay tests in BD Vacutainer (SST) serum tube with gel separator in reference with two different types of Barricor lithium heparin plasma tubes.

Methods: Blood specimens were collected from 50 subjects. Results comparisons for 62 tests were performed (33 chemistry and 28 immunoassays) using Architect chemistry and immunoassay analyzers, c8000 and i2000 respectively, in addition to osmolality test. By using the EP Evaluator system, the differences between SST and Barricor tubes (blue and grey stoppers) for each test were calculated using the error index (EI) and the allowable total error (TEa) values.

Results: By using the calculated EI, the difference between the old Barricor tube (blue stopper) and the SST gel tube was outside the TEa for LDH (46.0%), CA199 (48.5%), hsTrop-I (23.1%), CKMB (60.0%), C-peptide (14.3%) and Insulin (14.3%). After using the new Barricor tube with grey stopper, the differences were still outside the TEa for LDH (55.8%), CA199 (42.5%) and CKMB (23.8%).

Conclusion: Results from the old Barricor tube with blue stopper were generally comparable to the SST serum gel tube except in LDH, CA199, hsTrop-I, CKMB, C-peptide and Insulin tests. These differences were still unacceptable for LDH, CA199 and CKMB tests after using the new Barricor tube with grey stopper. Additional studies are needed to verify and determine the reasons for the unacceptable outcomes of comparability.

A-232

Serum Reference Interval Values of Selected Micronutrients, Vitamins, and Detectable Interleukins among Healthy Adults in South-Western Nigeria: A Preliminary Study

T. A. Adedeji[1], N. O. Adedeji[2], B. M. Fawale[3], O. A. Jeje[4], A. K. Ajeigbe[1], O. A. Smith[4], O. A. Ajose[1]. [1]*Department of Chemical Pathology, Obafemi Awolowo University, Ile-Ife, Nigeria,* [2]*Department of Obstetrics and Gynaecology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria,* [3]*Department of Medicine, Obafemi Awolowo University, Ile-Ife, Nigeria,* [4]*Department of Chemical Pathology, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria*

Background: Clinical decision making is largely influenced and mostly dependent on appropriate interpretation of numerical pathology reports. The laboratory results which form the bases of these reports are usually interpreted by comparison with the reference intervals. By inference, the quality of laboratory reports is therefore closely linked with the quality of the reference intervals used for their interpretation.

Methods: In this prospective study, a priori selection approach was used to select the subjects. The subjects were recruited among the clients of the blood-donating pool in the department of Haematology and blood transfusion unit of the hospital with age range: 18-64 years. They were screened for a panel of pathological conditions that could elicit acute systemic inflammation which could induce cytokine production.Serum micronutrients were assayed using Atomic Absorption Spectrophotometry (AAS), while serum interleukins and vitamins were assayed using High Performance Liquid Chromatography (HPLC). The 95% reference intervals (RIs) were estimated using reference limits at 2.5th percentile for the lower reference limit and 97.5th percentile for the upper reference limit. Reference intervals were derived using both parametric and non-parametric analysis for the purpose of comparison.

Results: One hundred and eighteen (118) apparently healthy subjects were selected. Their age ranged between 18-56 years with majority, 113 (95.8%) being aged 18-44years, and minority, 5 (4.2%) being between age 45-56 years. They were mostly males (blood donors), with only 13 (11.02%) being females, the remaining 105 (88.98%) were males. They were mostly Africans of Yoruba ethnicity which is one of major ethnic groups in South-west Nigeria. Estimated reference limits at 2.5th percentile for the lower reference limit and 97.5th percentile for the upper reference limit for the 5 micronutrients were as follows: Zinc: 9.49- 20.54 µmol/L, Selenium: 0.50-1.11 µmol/L, Copper: 13.86 - 27.97µmol/l, Iron: 14.19 - 32.07µmol/l, Manganese: 6.24 - 16.37nmol/l; for Magnesium: 0.78 -1.62mmol/l; For eight (8) selected vitamins: four

(4) fat-soluble vitamins (A, D, E and K), viz vitamin A: 1.08 -2.39 µmol/l, vitamin D: 59.89-164.42 µmol/l, vitamin E: 7.13-19.45µmol/l, vitamin K: 0.16-0.42nmol/l; and 4 water soluble vitamins (B1, B6, B12 and C) viz, , vitamin B1: 74.09 - 201.56nmol/l, vitamin B6: 0.12 - 0.29nmol/l, vitamin B12: 155.55 - 407.96nmol/l, vitamin C: 47.74 - 112.99 µmol/lAnd for 18 detectable/ detected interleukins (ILs) in the serum viz: IL-1: 0.58-1.24pg/ml, IL-2: 0.09-0.18pg/ml, IL-3: 0.39-0.89pg/ml, IL-4: 0.27-0.58pg/ml, IL-5: 0.08-0.19pg/ml, IL-6: 0.75-2.04pg/ml, IL-7: 0.37-0.96pg/ml, IL-8: 0.24-0.64pg/ml, IL-9: 0.49-1.29pg/ml, IL-10: 0.56-1.46pg/ml, IL-11: 0.27-0.58pg/ml, IL-12: 0.49-1.02pg/ml, IL-13: 0.04-0.07pg/ml, IL-14: 0.67-1.43pg/ml, IL-15: 0.07-0.12pg/ml, IL-16: 0.28-0.57pg/ml, IL-17:0.44-0.91pg/ml, IL-18: 0.74-1.56pg/ml.

Conclusion: There is an unparalleled agreement between our estimated reference interval values and those from other geographical climes, especially when compared with those in standard reports from well or almost perfectly planned studies (standardized 'a priori' selection studies), which thus promises the applicability of the reference values estimated for the selected micronutrients, selected vitamins, and detected interleukins in this study.

A-233

Improving Blood Culture Volume Compliance Rates for Both Nursing and Phlebotomy Teams by Multiple Educational Intervention Tools Across a Multihospital Health System

E. H. Zabaleta[1], P. Ozbolt[2], K. Krupinski-Shaw[2], S. Fuhrman[2], S. Antonara[2]. [1]*OhioHealth Mansfield Hospital, Mansfield, OH,* [2]*OhioHealth Riverside Methodist Hospital, Columbus, OH*

Background: Collection of adequate blood culture (BC) volume is the most important factor in blood culture positivity. It is a regulatory requirement to monitor the BC volume and provide feedback to collectors. In our multi-hospital health system, nurses collect BCs in the emergency departments (ED) and dedicated phlebotomist teams in other areas. We assessed the compliance to our blood culture volume policy and the effectiveness of various educational tools among collectors.

Methods: The optimal blood volume collected is defined as 8-10ml/bottle per hospital system policy and package insert. The blood volume of all aerobic bottles from each set was recorded utilizing the BacTec™ FX Epicenter software. Based on the individual volume of each draw, the instrument provides the mean volume for each collector along with the collection site information. We obtained the data for a 2 year period (12/2016-12/2018) in 4 separate month increments after educational interventions. Moreover, the compliance rates for optimal volume collection were calculated during the same time period.

Results: During the baseline period, the mean volume collected by phlebotomists at all sites was 5.1ml and compliance rate was 14.7%. For nurses (ED), volume and compliance was 5.2ml and 23.5% respectively. Educational intervention on the importance of blood culture volume collection took place which included both laboratory and nursing staff. During the post educational period, the mean volume collected by phlebotomists was 7.0ml and the compliance rate was 36.8%, while for nursing, it was 4.8ml and 20.3% respectively. Additionally, the data for individual phlebotomists were analyzed as part of a process improvement initiative led by laboratory management. The phlebotomists that complied with the policy were recognized and the ones that did not comply were coached on an individual basis. Furthermore, an educational tool was created for nurses and incorporated into their mandatory online training. After this intervention, the mean volume collected by phlebotomists was 7.8ml and the compliance rate was 51.9%, while for nurses it was 6.7ml and 32.3% respectively. To assess sustained compliance, six months after the last intervention, the phlebotomists had a mean volume of 7.8ml and compliance rate of 48.5% while nursing was at 7.1ml and at 39.1% respectively.

Conclusion: As a quality assurance measure, a compliance rate of 80% and a collection volume higher than 8ml was set for our phlebotomist teams. Utilizing the BacTec instrument volume function coupled with educational tools incorporated into required staff training, quality improvement measures and individual coaching led to increased volume compliance across the system. Nonetheless, our goal was not achieved and it is evident that continued education needs to occur to achieve and sustain optimal results.

A-234

Steroid Hormones

L. Duke[1], P. Kim[2], O. Sugahara[3], H. Zhou[1], C. Ulmer[1], H. Vesper[1]. [1]*CDC Atlanta, Atlanta, GA,* [2]*Battelle Memorial Institute, Atlanta, GA,* [3]*CDC Foundation, Atlanta, GA*

Background: Recent advancements in LC-MS/MS allow for the analysis of steroids with improved sensitivity and selectivity. In addition, the new sample preparation methodologies enable for steroid profiling in a quick and efficient manner. However, steroid measurements can be affected by pre-analytical factors such as the types of blood collection tube, sample handling, and storage temperature. Blood collection tubes used for serum and/or plasma analyses often contain surfactants and additives that may cause interferences, alter the recovery of analytes, and thus, cause pre-analytical variation. While the effect of the gel barrier in serum separation tube (SST) has been extensively investigated for drug measurements, little is known on the effect of serum separator gels on steroid hormone measurements. Lower abundant steroids and smaller serum volumes generally seem to be more affected by SST type. Therefore, this work investigates the suitability of multiple types of SSTs for reliable steroid measurements in serum.

Methods: Four types of SSTs with varying gels and additives were investigated for their potential impact on our ID LC-MS/MS method for progesterone (P4), estradiol (E2), estrone (E1), estrone sulfate (E1S), testosterone (TT), androstenedione (AD), dehydroepiandrostrerone sulfate (DHEAS), and 17-hydroxyprogesterone (17-OHP). Human off-the clot sera were split. One aliquot was measured directly and the second aliquot was stored in SST tubes over different time periods. Steroid hormones were isolated using liquid-liquid extraction and analyzed by UPLC/MS/MS. Chromatographic separation was performed using a phenyl-hexyl HPLC column with a C18 guard column. Mobile phases included methanol and methanol/ethanol/water. Electrospray ionization (ESI) was employed with alternating positive and negative ionization modes. Scheduled selected reaction monitoring (SRM) with two mass transitions (quantitation ion [QI] and confirmation ion [CI]) that are specific to the fragmentation of each steroid were used. Chromatograms, QI/CI ratios and results were compared between the original serum and the treated serum.

Results: The presence of new, non-resolved peaks, fluctuations in the chromatogram baseline, and deviations in QI/CI ratio were assessed to identify possible interferences resulting from the SST. Measurements were affected not only by tube type, but also by storage duration. Many SSTs affected steroid measurements partially due to an increased baseline, introduction of additional or interference peaks that can make integrations challenging. While results for 17-OHP and AD were not significantly different, substantial variances were observed for E1 and E1S, with an average decrease of 20%. Likewise, levels of TT and DHEAS increased by 35% and those of E2 by approximately 50%. Certain analyte measurements were further influenced by storage duration, including those for E1 and E2, for which deviations nearly doubled after an additional 16 hours of storage in some of the SST. P4 became unquantifiable due to a significant, coeluting interference peak when stored in certain SSTs.

Conclusion: Pre-analytical factors such as the type of SST can drastically impact the accuracy of certain steroid hormone measurements. Therefore, SSTs should be assessed for potential interferences before use to ensure accurate measurements.

A-235

Human Based Liquid Frozen Multi-Analyte Linearity Verification Material Covering Five Levels of Lipid Profile Components for Accurate Assessment of the Test System Reportable Range

S. O'Donovan, A. Shanks, L. Adams, M. L. Rodríguez, S. P. FitzGerald. *Randox Laboratories Ltd., Crumlin, United Kingdom*

Background: Calibration is the first step to ensuring reliable patient results. Calibration verification involves assaying materials of known concentrations in the same manner as patient samples to substantiate the instrument or test system's calibration across the reportable range as defined by Clinical Laboratory Improvement Amendments (CLIA). Stable, ready to use multi-analyte calibrators, covering at least five levels and meeting the requirements of individual analysers should be used to challenge the complete reportable range, therefore ensuring accurate patient testing. This study reports a human based multi-analyte verification material covering five levels of lipid profile components to facilitate the accurate diagnosis and treatment of lipid disorders.

Methods: Human based multi-analyte linearity verification material covering the lipid profile components: cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), at five levels. for each analyte, was manufactured. Values were assigned on the Roche Cobas C501 system. Open vial stability was de-

termined as the percentage recovery of each level thawed and stored 14 days at +2⁰C to +4⁰C related to a vial of the same material opened and thawed at day 0. Shelf life stability was determined by storing vials below -20°C and testing at regular intervals for up to 2 years.

Results: Assigned concentrations spanned the analytical range of the system, the ranges covered were: 0.6 to 18.5 mmol/L (cholesterol), 0.6 to 9.0 mmol/L (triglycerides), 0.2 to 3.3 mmol/L (HDL), 0.5 to 13.4mmol/L (LDL). The open vial stability assessment showed recovery values >97% (cholesterol),>97% (triglycerides), >93% (HDL), >96% (LDL) at all levels. Shelf life stability predicted: at least 2 years stored at -20°C to -80°C.

Conclusion: The reported multi-analyte verification material for lipid profile components is suitable for use to challenge the complete reportable range of the specified analyser to ensure accurate sample assessment to facilitate the accurate diagnosis and treatment of lipid disorders.

A-236

Reference Intervals Verification for Leukocytes, Erythrocytes and Epithelial Cells Proposed by Sysmex UF-5000 to Urine Tests in Southern Brazil Laboratory

N. Envall, D. Canali, A. Lopes, G. Pacheco, D. Faria. *Laboratório Médico Santa Luzia, São José, Brazil*

BACKGROUND: The reference intervals are fundamental in therapeutic and diagnostic evaluation. However, verify these values has been challenging in clinical laboratories. Within the possibilities to verify reference intervals, *a posteriori* test can be applied using laboratory database treated. This study verified reference intervals to urinary cell counts proposed by manufacturer.

METHODS: The urine samples were performed by Flow Cytometry method in Sysmex™ UF-5000 System. The test was performed based on the laboratory database results between May to October, 2018, from outpatient patients, over 18-year-old, both gender and showing normal results in urea, creatinine and urine culture. We used as statistical tool *EP Evaluator™ software, v. 19.0, USA*.

RESULTS: A total of 934 results were obtained following the inclusion criteria, without partition.

The median to Leukocytes, Erythrocytes and Epithelial Cells were 2,700/mL, 2,300/mL and 2,900/mL respectively. Using 95th percentile (confidence interval), the results observed to Leukocytes, Erythrocytes and Epithelial Cells were <14,328/mL, <15,200/mL and <23,000/mL respectively. The values proposed by manufacture, were < 25,000/mL, < 23,000/mL and < 31,000/mL in de same order.

CONCLUSION: Although the values showed had been adequate to local population, less tendency was observed when compared with the manufacturer. To this limitation, we proposed the reference intervals study *a priori.*

A-237

Performance Evaluation of the HIL Feature on the Atellica CH Analyzer

J. T. Snyder, E. Garcia, J. Davis. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: Endogenous interferences such as hemoglobin (H) from lysis of red blood cells; turbidity of insoluble lipids, or lipemia (L); and icterus (I) from endogenous bilirubin can be a significant source of analytical error for clinical chemistry and immunoassays. The Atellica® CH HIL Alert Index can notify the operator if HIL interference is present in concentrations high enough to affect assay results. HIL indices can be determined from dedicated HIL tests or from within the background of certain chemistry tests (ALT, AST, LDLP, and UN_c). This study evaluated the agreement of the Atellica CH HIL Alert Index feature with known concentrations of hemolysis, bilirubin, and INTRALIPID.

Methods: Samples were prepared for each index (0-6) by adding known amounts of hemoglobin, bilirubin, and INTRALIPID to normal human serum pools. Three replicates of each sample were processed on the Atellica® CH Analyzer with HIL, ALT, AST, LDLP, and UN_c. The mean HIL index result from the Atellica CH Analyzer was compared to the expected index for each sample.

Results: All hemoglobin and bilirubin sample results returned the expected index. All lipemic samples produced results within ±1 of the expected index value.

Conclusion: The results demonstrate acceptable agreement of the Atellica CH HIL Alert Index feature with known concentrations of hemolysis, bilirubin, and INTRALIPID.

Index	H (mg/dL)	I (mg/dL)	L (mg/dL)
0	≤10	≤1.9	≤124
1	11-130	2-9.9	125-249
2	131-249	10-19.9	250-499
3	250-499	20-29.9	500-699
4	500-749	30-39.9	700-999
5	750-999	40-59.9	1000-2999
6	≥1000	≥60	≥3000

A-238

Complete Depletion of Residual Therapeutic Monoclonal Antibody Interference in Serum Samples from Multiple Myeloma Patients to Improve Detection of Endogenous M-proteins

H. Vakili, S. Koorse Germans, X. Dong, R. Langevin, A. Muthukumar, I. Hashim. *UT Southwestern Medical Centre, Dallas, TX*

Background: Characterization and measurement of monoclonal protein is central to the diagnosis and follow up of multiple myeloma (MM) patients. Therapeutic IgG1 kappa humanized monoclonal antibody daratumumab (anti-CD38 antibody) is an FDA approved drug for the treatment of relapsed/refractory MM. Studies have identified a major interference of daratumumab in serum protein electrophoresis (SPEP) and immunofixation (IFE), which are the first line methods for identification of complete response by 'The International Myeloma Working Group' criteria. A more ominous threat to patient safety occurs with misinterpretation of a small daratumumab spike, as being the residual product of the patient's neoplastic clone, as oligoclonality or new clonality which could result in misinterpretation of failed to achieve remission. In this report, we describe a novel affinity-based magnetic beads to eliminate the false positive M-protein due to daratumumab reporting on SPEP and IFE with low cost reagents and negligible turn-around time with no requirement for dedicated instrument.

Methods: A donor serum with normal electrophoretic mobility (no endogenous M-proteins) was spiked with 0.5 g/L daratumumab. The spiked serum was incubated with 0.5 g/L of biotinylated recombinant CD38 (10 minutes incubation time) and streptavidin magnetic beads (5 minutes incubation time) and serum was separated on a magnetic stand and subsequently was run by electrophoresis (Sebia) according to the standard SPEP/IFE method. To demonstrate that the CD38 ligand antibody doesn't affect the endogenous M-protein migration and quantification, serum samples from 10 patients with MM with IgG kappa clone who were not receiving daratumumab as a therapy were spiked with 0.5 g/L daratumumab, and the impact of serum pre-treatment with beads-labeled CD38 were assessed on the quantification of endogenous M-spike (IgG kappa).

Results: Daratumumab spiking resulted in a clear IgG kappa M-protein on an IFE gel. However, incubation of daratumumab spiked serum with 0.5 g/L of biotinylated recombinant CD38 and magnetic beads successfully extracted the daratumumab with a magnetic stand and completely eliminated the IgG kappa signal on IFE gel. CD38 ligand antibody also did not affect the endogenous M-protein migration and quantification of all serum samples from patients with MM with IgG kappa clone that were spiked with 0.5 g/L daratumumab. Thus, this approach resulted in daratumumab-free serum samples and allowed for detection of true endogenous M-proteins.

Conclusion: Using a simple and low-cost sample treatment, we were able to deplete the monoclonal antibody daratumumab in serum from MM patients. This is a significant approach as a false positive interpretation of M-protein due to therapeutic monoclonal antibody such as daratumumab can obscure the detection of a second clone or a relapse. Therefore, additional unnecessary testing is done to follow up the patient and additional doses of cytotoxic therapy are warranted until complete response is achieved. Daratumumab extraction from serum using CD38 protein and magnetic beads can reduce excessive testing/treatment costs.

A-239

Can Chemistry Analytes be Measured Using Urine Collected in Urine Transport Tubes with Preservative?

D. Del Giudice, A. Adaya, D. B. Patel, H. K. Lee. *NorthShore University HealthSystem, Evanston, IL*

Background: The preferred collection container for measuring chemistry analytes in urine is a clear plastic tube with no preservative. In some occasions, physicians may want to add-on urine chemistry tests, but the only specimen available in the laboratory

is the urine in transport tube with preservative (Stockwell Scientific UrinalysisTube). The instructions for use (IFU) from the vendors often do not provide information on the acceptability of urine collected in urine transport tubes with preservatives for analysis.

Objectives: Primary objective was to determine if urine collected in urine transport tubes with preservative is suitable for testing twelve urine chemistry analytes. Secondary objective was to determine the stability of the twelve urine chemistry analytes in room temperature and refrigerated temperature over 72 hours.

Methods: Urine from sterile cups was aliquoted into 2 tubes without preservative and 2 tubes with preservative. One set of preservative and non-preservative tubes was stored at room temperature and all 12 analytes (amylase, calcium, chloride, creatinine, magnesium, microalbumin, phosphorus, potassium, sodium, total protein, urea nitrogen and uric acid) were tested at 0, 4, 8, 24, 48 and 72 hours. Another set of preservative and non-preservative tubes was stored at refrigerated temperature (4°C) and tested at 4, 8, 24, 48 and 72 hours.

Results: All analytes had percent recovery ranging from 93-107% except urine total protein, which showed a recovery of 80-87% between 4-72 hours post-collection. No significant difference in analyte concentration and stability were seen between urine stored in preservative versus non-preservative tubes for 72 hours post-collection.

Conclusion: Urine collected in Stockwell Scientific UrinalysisTubes can be used to test the 12 analytes studied, with acceptable recovery within 72 hours of collection, at room temperature or refrigerated temperature.

A-240

How High Can We Go? Expanding the Reporting Range of Ferritin to Facilitate the Acute Diagnosis of Hemophagocytic Lymphohistiocytosis

H. A. Paul, V. A. Allison, J. L. Gifford. *Alberta Public Laboratories, Calgary, AB, Canada*

Background: Hemophagocytic lymphohistiocytosis (HLH) is characterized by an aggressive activation of the immune system that can lead to widespread tissue damage, multi-organ failure, and sometimes death. HLH most often affects children up to 18 months of age and is triggered by events that stress and/or disrupt the immune system, such as infection. The diagnosis of HLH includes serum or plasma ferritin concentration >500µg/L. Ferritin concentration >10,000µg/L occurs almost exclusively in HLH, therefore the ability to report ferritin >10,000µg/L is important in its diagnosis. Ferritin at Alberta Public Laboratories in Calgary was previously measured using a Roche Diagnostics electrochemiluminescence immunoassay (cobas e 601; clinical reportable range (CRR) up to 100,000µg/L for 50-fold diluted samples). Ferritin is now measured using the immunoturbidimetric Tina-quant Ferritin reagent (cobas c 701; CRR up to 8000µg/L for 8-fold diluted samples). This change resulted in numerous urgent clinical inquiries from rheumatologists requiring the concentration of ferritin above the CRR, specifically for the diagnosis of HLH. This study aimed to support the acute diagnosis of HLH by expanding the CRR of the Roche Diagnostics Tina-quant Ferritin Gen.4 assay via dilution verification.

Methods: Patient plasma samples (N=13) with ferritin concentrations near the upper limit of the analytical measuring range (1000µg/L) were identified using Roche Cobas middleware (mean 948ug/L; range 908-980µg/L). Manual (5, 10, 20, 50 and 100-fold) and on-board (5, 10, 20, and 50-fold) dilutions were performed using Diluent NaCl 9%, the manufacturer's recommended diluent for the Tina-quant Ferritin Gen.4 reagent. Manual (5, 10, 20, 50 and 100-fold) dilutions were also performed with Roche Diluent Universal, the recommended diluent for the previous electrochemiluminescence immunoassay. Results were assessed using bias plots, and dilutions were determined to be valid if 95% of the results were within the biological variation-derived desired total allowable error (TAE) of this assay (±17%) when compared to the neat values. Student's t-test assessed differences (significance at $p<0.05$).

Results: On-board dilutions with Diluent NaCl 9% (mean 7%, 95% CI 5-9%, range -5-20%) produced less variability and significantly smaller bias in results compared with manual dilutions (manual Diluent NaCl 9% mean 18%, p=0.007, range: -14-140%; manual Diluent Universal mean 15%, p=0.007, range -14-127%). The 50-fold on-board dilution had 95% of results within the target TAE (mean 7%, 95% CI 3-11%, range -4-18%). The 100-fold manual dilution with Diluent NaCl 9% had a range of -14 to 28%, with 85% of the results within the target TAE. A reporting workflow based on automated and manual diluting protocols was developed.

Conclusion: The CRR of the Tina-quant Ferritin Gen.4 reagent can be increased to 50,000ug/L with a 50-fold on-board dilution with Diluent NaCl 9%. In addition, clinical requests for quantifying results between 50,000 and 100,000ug/L may be reported. This wider, analytically acceptable CRR has improved patient care and better meets the needs of rheumatologists.

A-241

Laboratory Protocol for the Identification of Estradiol (E2) and Unconjugated Estriol (uE3) Interference on the Beckman UniCel DXI 800 Immunoassay System

S. P. Wyness[1], A. S. Maharjan[2], J. A. Ray[1], T. L. Willcox[3], J. D. Seiter[3], J. R. Genzen[2]. [1]*ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT,* [2]*Department of Pathology, University of Utah, Salt Lake City, UT,* [3]*ARUP Laboratories, Salt Lake City, UT*

Background: Estradiol (E2) and unconjugated estriol (uE3) on the UniCel DXI 800 immunoassay system (Beckman Coulter; Brea, CA) are competitive immunoassays. On rare occasions, a "No Value" instrument error flag may be obtained, which occurs when the numeric relative light unit (RLU) result is beyond the RLU of the zero calibrator. Once potential instrument malfunction has been excluded, this finding may represent a true low analyte concentration below the assay analytical measuring range (AMR) or alternatively assay interference. Our laboratory has recently characterized interference for E2 and uE3 assays due to endogenous antibodies against bovine alkaline phosphatase, a reagent component in these competitive immunoassays. The objective of the present study was to develop a simple protocol for the differentiation of "No Value" results due to true low analyte concentrations (which can be reported as < AMR) from those caused by endogenous assay interference.

Methods: Seven materials – Access Sample Diluent A (Beckman), Access Wash Buffer II (Beckman), Access Unconjugated Estriol Zero Calibrator (Beckman), Access Estradiol Zero Calibrator (Beckman), Access Red Blood Cell Folate Lysing Agent (Beckman), Saline (Roche), and demineralized distilled H_2O (General Water Technologies) – were evaluated as potential diluents by investigating baseline E2 and uE3 quantitative results and their potential for matrix interference. Using archived clinical specimens with baseline "No Value" E2 or uE3 results, dilution strategies were evaluated by comparing the DXI results to corresponding quantitative E2 or qualitative uE3 measurements by an in-house, high performance liquid chromatography - tandem mass spectrometry (LC-MS/MS) method. A dilution strategy was developed and then tested on additional clinical specimens with baseline "No Value" E2 or uE3 results.

Results: Beckman Sample Diluent A was identified as the optimal protocol diluent, as baseline E2 and uE3 measurements produced "quantitative" results below the assay AMR but without E2 or uE3 "No Value" interference. Excellent recovery (100±15%) of spiked analyte into Sample Diluent A was observed for uE3 (x2, x5, and x11 dilutions). While under-recovery of spiked analyte into Sample Diluent A was observed for E2 (x2, 70%; x5, 50%; x11 dilutions, 40%), this under-recovery did not interfere with its use in subsequent interference-exclusion protocols. However, sample Diluent A should not be used for the reporting of quantitative (within AMR) results due to risk of under-recovery. A 1:2 dilution protocol for "No Value" patient specimens (100 µl patient specimen + 100 µl Sample Diluent A) proved effective at differentiating scenarios where "No Value" flags for E2 and/or uE3 were due to true low concentrations (verified by LC-MS/MS) versus endogenous assay interference.

Conclusion: A laboratory protocol for the differentiation of "No Value" E2 and uE3 results due to true low analyte concentration versus endogenous interference has been developed. This protocol has enabled a safe method for reporting true low (e.g. <AMR) results when identified.

A-242

Data Mining of 2 Years of Stat and Emergency Testing in Multiple Institutions Demonstrates Significant Blood Drawing Tube Dependent Preanalytic Errors in Hemoglobin or Hematocrit Measurements

E. Xu[1], G. Cembrowski[2], H. Sadrzadeh[3], A. Fuezery[4], R. Schifman[5]. [1]*University of Manitoba, Winnipeg, MB, Canada,* [2]*University of Alberta, CC Quality Control Consulting, Edmonton, AB, Canada,* [3]*Calgary Laboratory Services, Calgary, AB, Canada,* [4]*Alberta Health Services, Edmonton, AB, Canada,* [5]*University of Arizona, Veterans Administration Hospital, Tucson, AZ*

Introduction: Point of care (POC) hemoglobin or hematocrit can include significant preanalytic variation secondary to inadequate specimen mixing prior to analysis. Three different POC hemoglobin/hematocrit measurement systems were evaluated, 1) Radiometer ABL800 with blood drawn into the SafePico tube containing a metal ball facilitating blood mixing by the blood drawer and/or the ABL800, 2) Instrumentation Laboratory GEM 4000 with blood drawn into the SafePico tube without instrument-based mixing 3) Abbott iSTAT with blood pipetted from the blood drawing tube. Serial intrapatient measurements were transformed into measures of preanalytic and analytic variation.

Methods and Materials: Serial hemoglobins or hematocrits were obtained from Abbott iSTATs operated in 15 US VA hospitals, Radiometer ABL800s analyzing venous blood gases (VBG) in 5 Edmonton hospital emergency departments (ED) and tandem GEM 4000s analyzing VBG from the Calgary Foothills Hospital ED and ICUs. For hemoglobin or hematocrit, we tabulated consecutive pairs of intra-patient results separated by time intervals of 0-2 ,2-4, 4-6, up to 12 hours. The average between pair variations were regressed against time with the y-intercept representing short term analytic variation and preanalytic (pa) variation including biologic variation: $y_o^2 = s_a^2 + pa^2$ (ClinBiochem 2017;50:936-941)

Results: The Table summarizes the study results including a 2015 study of outpatients analyzed in a referral laboratory. The lowest variation is Radiometer/SafePico and the highest is Abbott iSTAT.

Discussion: Total allowable error for hemoglobin and hematocrit is 4% and is based on normal intrasubject variation of 3%. ED patients have fluid and blood loss and are often treated with fluids and blood with their hemoglobins and hematocrits varying by more than 3% in a 12 hour period. Radiometer/SafePico variation thus seems optimal; iSTAT's overly high hematocrit variation is mostly due to insufficient mixing. If the iSTAT user used the safePICO tube, they would achieve variation close to GEM's.

Instrument	Analyte	Specimen Pairs	Patient Mean	yo	Total Variation
Abbott iSTAT	Hematocrit, fraction	9234	0.39	0.047	12.1%
IL Gem 4000	Hemoglobin, g/dL	5964	12.0	0.96	8.0%
Radiometer ABL 800	Hematocrit, fraction	9059	0.39	0.024	6.2%
Referral Lab (2015)	Hemoglobin, g/dL	------	13	0.32	2.5%

A-243

When a Dried Sample Isn't Dry: How Different Sample Handling Procedures between the Clinic and the Analytical Laboratory Can Impact Drug Quantification

A. P. Schauer, C. Sykes, K. H. Blake, A. D. M. Kashuba. *University of North Carolina, Chapel Hill, NC*

Background: When monitoring drug adherence, there are advantages in the collection of whole blood by volumetric absorptive microsamplers (VAMS) or dried blood spots (DBS) compared to collecting blood plasma by phlebotomy. Dried sampling is less invasive (collected by finger stick rather than venipuncture) and requires smaller volumes (benefiting pediatrics and geriatrics). Here we describe results from a clinical study quantifying the antiretroviral dolutegravir (DTG) in human whole blood via VAMS and DBS where sample handling differed in the clinic and the analytical laboratory.

Method:12 subjects dosed to steady state with DTG had 180 paired blood samples collected over 28 days by VAMS (wicked on a 20uL Mitra® tip) and DBS (spotted 50uL on a Whatman 903 card). Samples dried for 2-5 hours. Following sample analysis by LC-MS/MS, the DTG concentrations from VAMS and DBS were found to be disparate. It was then recognized that the clinical VAMS samples were placed into a 2mL tube with the lid uncapped to dry, while the VAMS samples used for calibration standards and quality controls (QC) in the laboratory were left in the clamshell (Neoteryx package which holds the VAMS microsamplers) device to dry upright. DBS sample preparation was identical between the clinic and laboratory. A comparison of two different methods of drying the VAMS samples was performed at a DTG concentration of 100ng/mL. VAMS samples were left out to dry (uncapped in a tube and in the clamshell) for 2, 3, 4, 5 hours to mimic clinic and laboratory procedures and 24 hours to facilitate complete drying before being stored at -80°C. A parallel evaluation was performed with DBS, using one drying method (lying flat) only, which both the clinic and laboratory utilized. Data are presented as mean (standard deviation). Calculations were performed with Excel.

Results: Whereas the whole blood samples via DBS and VAMS should have had identical concentrations, DTG concentrations in the VAMS clinic samples were 1.9 (0.43)-fold higher than its paired DBS sample. Our exploratory test results showed a 1.8 (0.07) [VAMS in Tube:Clamshell] and 2.0 (0.24) [VAMS in Tube:DBS] fold increase in DTG response for the 2-5 hour dry time, mimicking what we saw in the clinical samples. By observation, VAMS stored in the tube for up to 5 hours were not dry, while VAMS in the clamshell were dry within 2 hours. The coefficient of variation of DTG response for the 2-5 hour time points was 6% [VAMS-Clamshell], 4% [VAMS-Tube], and 11% [DBS] demonstrating minimal alteration in concentration for all samples drying between 2-5 hours. The VAMS samples were completely dry by both methods at 24 hours, and yielded similar concentrations (within 11%) to DBS, suggesting our discrepancy was a result of sample dryness.

Conclusion: These results explain, as a result of incomplete drying of VAMS in the clinic, why DTG concentrations were 2-fold higher than DBS. When working with VAMS, it is important to ensure that the clinic and laboratory protocols are similar because extraction efficiencies can differ between wet and completely dried samples.

A-244

Chemistry Standardization across a Network: The MSK Experience

P. Erdman, E. Schlauch, A. Ocasio, M. Pessin, L. Ramanathan. *Memorial Sloan Kettering Cancer Center, New York, NY*

Background: Standardization is important to quality patient care due to the emergence of health care systems having multiple laboratory sites. Memorial Sloan Kettering Cancer Center (MSKCC) has a commitment to create "One MSK" where all patients receive the same level of care regardless of where they are treated. This is especially important for MSK as our patients travel around the NYC metro area. We describe our process of creating a standardized clinical laboratory network across nine laboratory sites with continuous monitoring, servicing both inpatients and outpatients. **Methods:** We have 16 Abbott Architect instruments (10 c4000's, 4 8000c's and 2 c16000c's). Daily, weekly and monthly maintenance procedures are standardized. We have standardized our lots of Bio-Rad QC across all sites, but not yet our reagents. All sites join a "weekly WebEx" to ascertain if there are any instrument or other issues that need to be discussed. Data was extracted from the following sources: internal MSKCC de-identified patient QC data, Biorad Unity QC reports, CAP PT program data, Maine Standards linearity data, and Cerner LIS. We analyzed patient data to determine how often our patients move between clinical sites. For analysis purposes, our core laboratory analyzers were considered "reference" instruments. Intra- and interlaboratory instrument correlations were conducted on a monthly basis using anywhere from 10-50 patient samples for each analyte. The cumulative results were also evaluated on a 6-month basis. Total allowable error (TEa) was established using Westgard standards. **Results:** Monthly instrument correlations spanned a broad analytical range for each analyte, all with acceptable TEa's over a two-year period. Biorad QC results were analyzed from August-December 2018 across all laboratories. The values for MULT-1, MULT-2, and MULT-3 were averaged together for 5 months. The first value provides the MSKCC group CV and the second value provides the CV for the entire method group. The values are: Albumin-1.5%/3.1%, AST-2.3%/6.9%, ALT-3.5%/7.2%, Amylase-2.1%/10.7%, BUN-2.8%/13.5%, Calcium-1.3%/2.0%, Chloride-0.7%/2.4%, CO2-6.7%/8.4%, Creatinine-3.2%/5.9%, Direct Bilirubin-1.6%/20.2%, Glucose-1.5%/2.7%, Lipase-4.3%/4.7%, Magnesium-4.8%/4.8%, Phosphorus-2.1%/3.6%, Potassium-1.0%/2.2%, Sodium-0.7%/1.3%, Total Bilirubin-1.6%/6%, Total Protein-1.2%/2.4%, and Uric Acid-1.8%/2.9%. CAP PT data was examined using the "General Chemistry/Therapeutic Drug Survey". In 2017-2018, for surveys A, B, and C combined, the total score for all assays was 100%. Individual laboratory SDI's were consistently below peer SDI's. Six-month linearities reliably covered a wide analytical range, and all sites implemented the same reportable ranges and reference ranges. The laboratory TAT performance for basic chemistries (BMP and CMP) in 2018 was less than 60 minutes for both inpatients (98%) and outpatients (99%). Review of Cerner LIS data demonstrated that approximately 15% of our patients are seen at more than one clinical site. A review of 20 patients who had been seen at more than one site found almost no difference between sites for a given patient's Creatinine or Total Bilirubin level. **Conclusion:** Our data show that a robust program to standardize instruments across multiple clinical sites can be created to provide high quality patient care. We are confident that our patients will have reproducible results where ever they present for care within our institution.

A-245

Effect of Freeze-Thaw on Serum HDL-Cholesterol and LDL-Cholesterol Concentrations Determined by Ultracentrifugation-and Electrophoresis-based Methods

K. Dahya[1], B. Smith[2], D. Lazare[1], U. Danilenko[1], H. W. Vesper[1]. *[1]CDC, Atlanta, GA, [2]Battelle, Atlanta, GA*

Background: Pre-analytical conditions affect the accuracy of low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) testing. Freezing with consecutive thawing was suggested to result in re-distribution of LDL-C and HDL-C and therefore might change the measured concentrations. Some studies found that even one freeze-thaw cycle may cause up to 37% changes in HDL-C and LDL-C concentrations measured by a modified ultracentrifugation-based procedure, while other studies reported no significant changes. The aim of this study was to investigate further the impact of freezing on blood lipids measurements and highlight the importance of pre-analytical factors in LDL-C and HDL-C testing.

Methods: HDL-C and LDL-C were measured in 20 fresh, never frozen single donor serum samples using the CDC ultracentrifugation-based reference measurement procedure (CDC RMP). In brief, aliquots of serum were ultracentrifuged at d 1.006 kg/L. The top fraction was removed and the remaining bottom fraction (BF) cholesterol was collected quantitatively. HDL-C was measured separately by precipitation after recon-

stituting the BF. The LDL-C was precipitated from the BF-C with heparin-manganese chloride to obtain the HDL-C fraction. The cholesterol content in the BF and in the HDL fractions were measured by the Abell-Kendall RMP in 4 replicates and LDL-C was calculated by subtracting the HDL-C from the BF-C. The serum aliquots underwent one freeze/thaw cycle and analyzed again. In addition, the same sets of fresh and once-frozen samples were analyzed using the LDL Lipoprint subfractions assay (Quantimetrix). Lipoprint gel electrophoresis was performed in duplicates. The differences between fresh samples and once-frozen samples were expressed as percent bias. **Results:** Serum samples had HDL-C concentrations ranging from 33.61 mg/dL to 145.00 mg/dL and LDL-C concentrations from 51.85 mg/dL to 146.03 mg/dL. Based on the CDC RMP, the percent bias between fresh and frozen HDL-C results ranged from -10.7% to 4.2%. The bias was not dependent on the HDL-C concentration or triglycerides levels. Only 2 of 20 samples had bias outside the National Cholesterol Education Program (NCEP) recommended criterion of 5%. The percent bias for fresh vs. frozen samples ranged from -9.2 to 20.3%. Changes caused by freezing were not consistent between HDL-C measured by Lipoprint and CDC RMP. For LDL-C measurements, the percent bias ranged from -7.4% to 12.3 %, with 6 out of 20 samples being outside of the ±4% NCEP criterion for bias. No correlation with LDL-C or triglycerides concentration levels was observed. The differences between fresh and frozen samples measured by the Lipoprint technology ranged from -3.85% to 9.64% with only 3 samples being outside of the ±4% bias criterion. A shift in lipoprotein particle distribution was observed in several serum samples after freezing.

Conclusion: Freezing introduces increase or decrease in HDL-C and LDL-C concentrations in matching serum samples. Data suggest that the impact of freezing cannot be predicted and is independent of the initial HDL-C, LDL-C or triglycerides concentrations. The potential changes in HDL-C and LDL-C should be considered when conducting clinical research studies using frozen samples.

A-246

Antigen Excess due to Extreme Hyperferritinemia following CART Therapy

L. M. Moore, D. Carlow, K. Murata. *MSKCC, NEW YORK, NY*

Background: Chimeric Antigen Receptor T-cell (CAR-T) therapy is a novel treatment modality that uses genetically modified T-cells, capable of targeting T-cell receptors that are specific to certain tumor antigens. While effective, the use of CAR-T therapy is known to be associated with Cytokine Release Syndrome (CRS). CRS is a potentially life-threatening adverse side effect caused by massive T-cell activation. This T-cell activation initiates a pro-inflammatory cytokine cascade, which is often associated with an increase in serum acute phase reactants (APR), such as ferritin. In this study, the laboratory director was contacted by a physician regarding inconsistent ferritin results for a patient who had recently undergone CAR-T therapy. The physician anticipated that ferritin would rise, but instead observed a decrease in ferritin values (<1500 ng/mL). After investigation it was discovered that the patient had extreme hyperferritinemia (400,000 ng/mL). The excess ferritin in the patient's sample exceeded the capacity of our immunoassay. This led to the reporting of a falsely low serum ferritin concentration; a phenomenon known as the hook effect. **Objective:** The main objective of this study was to assess ferritin excess on four different chemistry analyzers to compare susceptibility to the hook effect. **Methods:** Ten serum samples were collected during routine clinical testing for the same patient. Ferritin measurements were obtained using the following instrumentation: The Siemens Immulite, Siemens Centaur, Ortho Clinical Diagnostics Vitros 5600, and the Roche E170. All specimens were run twice on each instrument; neat and diluted. Diluted specimens were serially diluted until corrected ferritin concentration stabilized. Dilution factors ranged from 50X - 500x. **Results:** When the samples were run neat, without prior off-line dilution, only the Ortho Vitros 5600 and the Roche E170 correctly reported the specimens to have a ferritin level beyond the analytical measurable range (AMR); while the Siemens Immulite and Centaur instrumentation reported falsely low results that fell within the instrument's AMR. Extremely high serum ferritin levels were only reportable when specimens underwent pre-dilution prior to analysis. The ferritin measurements obtained during this study ranged from 80,000 ng/mL - 400,000 ng/mL among the different instrumentations. The highest ferritin levels were observed at 5-days post administration of CAR-T therapy. **Conclusions:** Our data has shown that the Siemens Immulite and Centaur are most susceptible to the ferritin excess hook effect. This susceptibility to the hook effect is likely due to differences in the manufacturer's protocol and/or AMR. To our knowledge this is the first reported case of hook effect due to extreme hyperferritinemia following CAR-T therapy. With the rise of CAR-T therapy, it will be imperative for labs to re-evaluate the clinically reportable range for serum ferritin and other analytes that may increase with CAR-T therapy-induced CRS.

A-247

The Use of Mitotic Stimulants in Cell Culture for Karyotyping in Cytogenetics Analyses

G. D. Lima, M. Conchon, J. M. Fenandes. *DASA, Barueri, Brazil*

Background: In order to perform the cytogenetic examination it is necessary to use some stimulants, so that cell division can occur in vitro. Among the main stimulants phytohemagglutinin (**PHA**) is used, in other cases the use of Phorbol 12-myristate 13-acetate (**TPA**) is more appropriate.

Objective: Verify the efficacy of mitotic stimulants in peripheral blood and bone marrow cell culture.

Methods: As a medium culture, we used a complete medium enriched and supplemented by RPMI 1640, fetal bovine serum, L-glutamine, penicillin and streptomycin. For heparinized peripheral blood samples with diagnostic hypothesis of constitutional diseases, the stimulator used was **PHA**. For bone marrow punctures with sodium anticoagulant heparin, with diagnostic hypothesis of lymphoma, myeloma multiple, plasmocytosis, *etc*, the stimulator was **TPA.**

Results: With the addition of these stimulants, we can provide a significant increase in the mitotic index, verified by means of an optical microscope.

Conclusion: In order to perform a chromosome analysis, cells must be able to grow and divide rapidly into culture media. The first step to perform a culture cell process is the material collection and is determinant for its success. For culture cells in vitro growth, it is necessary to have a culture medium that approaches the in vivo environment in relation to temperature, oxygen and carbon dioxide concentration, pH, osmolarity and nutrient supply. With all these factors being possible to obtain the metaphases.

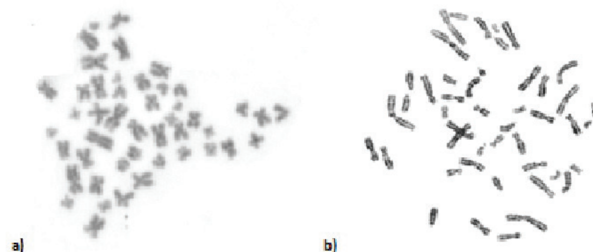


Figure 1. a) Karyotype of bone marrow with TPA, **b)** karyotype of peripheral blood with PHA.

A-248

Orthogonal Confirmation for NGS should not be Completely Eliminated into Clinical Practice for Amplicon-Based NGS Panels

M. A. Pereira[1], P. G. P. Couto[2], N. P. Lopes[2], J. P. Silva[1], A. A. Morais[2], R. L. M. Guedes[1], D. Zauli[1]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Progenética Laboratory), Belo Horizonte, Brazil*

Background: The debate regarding the necessity of NGS confirmation is still conflicting. International guidelines for NGS validation do not present a joint consensus recommendation and clinical laboratories determine individually whether confirmation testing is appropriate. Furthermore, many laboratories do not have resources for secondary confirmation due to additional costs. Some research groups evaluated the need for Sanger confirmation and Wenbo Mu and colleagues (2016) concluded that quality thresholds for high-confidence NGS calls, and Sanger sequencing for low quality variants could ensure the highest sensitivity and sensibility of the assay decreasing false-positive (FP) results. However, it is still challenging to discern between sequencing errors without secondary confirmation. **Objective:** Describe sequencing errors deriving from high-quality regions in NGS with Amplicon-based panels. **Methodology:** Two custom Ion AmpliSeq panels for mutation screening of *BRCA1/2* and *TSC1/2* were analyzed. Genomic DNA libraries were prepared from blood samples, following the manufacturer's instructions. Sequencing was performed on the Ion S5™ platform. Signal processing, base calling and sequence alignment against the hg19/GRCh37 reference genome were performed on Torrent Suite™ 5.6 Server and variant calling by Ion Reporter™ 5.6 Software. Exonic and exon-intron junction variants were analyzed and classified according to the ACMG/AMP variant interpreta-

tion recommendations. Variants were manually inspected with Integrative Genomics Viewer (IGV) visualization. Point mutations or small insertions/deletions classified as pathogenic, probably pathogenic and occasionally VUS were confirmed by Sanger sequencing. **Results and Discussion:** One variant (*BRCA2*, LRG_293t1:c.956dupA, Asn319Lysfs*8, rs80359770, pathogenic, MAF=8.7e-0.6) observed in a homopolymer region was found to be true-positive (TP). Since it is commonly detected in several patients, it could be considered as technical artifact. Another heterozygous variant (*TSC2*, LRG_487t1:c.5161-28_5161-25del, rs1799758, VUS, MAF=0.0086) was found to occur in one patient at the primer 3' end, leading to single allele amplification and FP homozygous calls of two heterozygous variants in this amplicon. Finally, one *BRCA1* variant (LRG_292t1:c.4964_4982del, p.Ser1655Tyrfs*16, rs80359876, pathogenic, MAF=8.2e-0.6) detected in two patients was detected as false-negative (FN) after MLPA analysis (P002 BRCA1 and P087 BRCA1 Confirmation probemixes; MRC-Holland) and Sanger sequencing. This deletion is localized in both extremities of NGS amplicons covering the region, impairing the alignment and consequently, the variant call. **Conclusions:** This study demonstrates errors affecting Amplicon-based NGS sequencing and reinforces the importance of secondary confirmation. Since rare variants affected amplicon efficiency, common, unknown and/or population-specific polymorphisms could have a greater influence in clinical routine. These examples would easily pass automatic high-quality control filters, such as high sequencing coverage, expected germline allele frequency and mapping quality. Orthogonal confirmation can reduce the chances of delivering a FP or FN results and ensure an accurate and informative report for clinical management and treatment decisions by healthcare providers.

A-249

High Serum CPK Level as an Analytical Interfering Potential in the Evaluation of Transaminases

M. C. G. CASTELO[1], L. L. A. Cavalcante[1], T. A. C. Palhano[1], S. P. Bandeira[1], C. S. Candido[1], F. L. B. Furtado[2], G. A. Campana[3]. *[1]DASA, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]DASA, São Paulo, Brazil*

Objective: To describe possible analytical interference of elevated levels of CPK in the analysis of transaminases (AST and ALT) in the absence of hepatic damage. Clinical case: Patient, 47 years old, male, diagnosis of mixed primary dyslipidemia 5 years ago, using statin (rosuvastatin) and fibrate (fenofibrate). Refers a proximal myalgia, upper limbs, of sudden onset, after moderate ingestion of alcohol, which led him to emergency service. Initial examinations showed CPK 15879 U/L (kinetic UV method), AST 666 U/L (kinetic UV method) and ALT 352 U/L (kinetic UV method). The clinical management was carried out at home with hydration and suspension of the medications. The decrease in CPK was rapid and progressive and accompanied by concomitant normalization of transaminases in the short time interval (Table 1). The CPK enzyme is the most specific and sensitive laboratory test for the assessment of muscle damage. Among the pre-analytical factors that may act as potential analytical interferers in this case, we highlight the exposure to alcohol, use of statin (rosuvastatin) and fibrate (fenofibrate). AST is also a muscle enzyme and could be elevated in the presence of myopathy, however the ALT should not be altered in this condition. An elevation of ALT of more than 10-fold, as occurred in this case, may raise the presence of liver damage. However, extensive laboratory evaluation showed normal viral serology and autoimmunity panel associated with normal imaging (bile duct and liver). These facts made the hypothesis of liver disease unlikely. It is important to emphasize the abrupt fall of the transaminases concomitant to the reduction of CPK, a recovery that would probably not occur if there was actual hepatic damage. The present report ratifies the importance of medical knowledge with regard to the preanalytic, analytical and post-analytical interferences of clinical practice.

Table 1

Date	CPK (U/L)* R°I: ≤ 171	AST (U/L)* RI: < 50	ALT (U/L)* RI: < 50
13/01/18	68	26	37
22/06/18	50	21	33
07/07/18	15879	666	352
09/07/18	844	135	135
14/07/18	67	25	25
11/08/18	59	22	22
06/10/18	65	21	30
10/11/18	62	22	29
°: reference interval *Method: Kinect UV			

A-250

The Usefulness of Postprandial a Glucose Spike Inhibiting by MCT Oil

T. Nakahara, H. Kataoka, K. Tohyama. *Kawasaki University of Medical Welfare, Kurashiki, Okayama, Japan*

Background: Medium-chain triglycerides (MCT) are composed of a glycerol backbone and three fatty acids that have an aliphatic tail of 6-12 carbon atoms. MCT oil is easily digested and absorbed by the body. Moreover, it efficiently restricts carbohydrates by producing ketone bodies and does not easily accumulate in the body. In general, blood glucose and triglyceride levels rise after meals. However, MCT oil inhibits the rise in blood glucose levels. **Aim**: This study aimed to investigate the inhibitory effect of MCT oil on postprandial rise in blood glucose levels using flash glucose monitoring (FGM). **Methods**: A continuous glucose monitoring device (FreeStyle Libre FGM system) was attached to the upper arm of 6 adult volunteers (4 men and 2 women) in their 20s-60s, and the readings on the glucose levels were recorded. The glucose levels were measured for 3 days in a group in which the subjects took 15 mL of MCT oil only before breakfast and had subsequent breakfast, lunch, and dinner as usual and in a group in which the subjects took 5 mL of MCT oil before breakfast, lunch, and dinner. The 5-mL and 15-mL MCT oil groups were individually compared to a 0-mL MCT oil group in which the subjects did not take MCT oil. **Results**: When the subjects took MCT oil, a rise in postprandial blood glucose levels was inhibited by a maximum of 30.2% compared to when the subjects did not take MCT oil. When 15 mL of MCT oil was taken only before breakfast, the mean inhibition rates were (mean ± SD) 19.6 ± 6.1%, 5.7 ± 12.6%, and 1.8 ± 17.7% after breakfast, lunch, and dinner, respectively. When 5 mL of MCT oil was taken before every meal, the mean inhibition rates were 11.9 ± 12.2%, 12.0 ± 16.0%, and 8.7 ± 10.6% after breakfast, lunch, and dinner, respectively. Moreover, although a greater degree of inhibition of rise in blood glucose levels was observed when 5 mL was taken before every meal compared to when 15 mL was taken only before breakfast, the inhibitory effect was maintained all day long in both groups. **Discussion**: A postprandial rise in blood glucose (a glucose spike) levels may trigger myocardial infarction or stroke. Moreover, a postprandial rise in blood glucose levels increases diabetes risk even in healthy individuals. Taking MCT oil may be useful in reducing these risks. **Conclusion**: In all subjects, it was confirmed that MCT oil had an inhibitory effect of about 5%-20% on postprandial rise in blood glucose levels.

A-251

Barricor Blood Drawing Tube Reduces Preanalytic Variation in Beckman Coulter AccuTnI+3 Assay: A Natural Experiment

G. Cembrowski[1], E. Xu[2], A. Tsui[3], J. Raizman[3], A. Fuezery[3]. *[1]University of Alberta, CC Quality Control Consulting, Edmonton, AB, Canada, [2]University of Manitoba, Winnipeg, MB, Canada, [3]University of Alberta, Edmonton, AB, Canada*

Introduction: The Beckman Coulter AccuTnI+3 assay produces sporadic false positive elevations in cardiac troponin I, secondary to cellular debris, e.g. platelets. In a limited study our group showed that the Barricor, a new Becton Dickinson (Franklin Hills, NJ) blood collection tube using a novel mechanical separator appeared to mitigate spurious, nonreproducible false elevations in cTnI. In this work, we describe a retrospective analysis of intrapatient variations of troponins before and after Barricor's implementation in 2 large Edmonton hospitals and demonstrate significant reduction in preanalytic variation in troponin measurements.

Methods and Materials: From a laboratory data repository, we obtained from Edmonton's 2 largest hospitals, 2.0 and 0,75 years of AccuTnI+3 plasma troponin results from before and after the implementation of the Barricor tube, respectively. For each data set and 4 troponin concentration ranges we tabulated consecutive pairs of intra-patient troponins separated by time intervals of 0-1 ,1-2, 2-3, up to 12 hours. The average between pair variations were regressed against time with the y-intercept representing short term analytic variation(sa) and preanalytic (pa) variation including biologic variation: $y_o^2 = s_a^2 + pa^2 + s_b^2$ (ClinBiochem 2017;50:936-941).

Results: The Table summarizes the differences in the y-intercepts for the 4 different troponin ranges and the reductions in preanalytical variation associated with the use of the Barricor tube.

Discussion: At the level of 100 ng/L, a current cutoff for the the AccuTnI+3, the Barricor blood drawing will reduce the preanalytical variation of the troponin by around 3%, more so at high concentrations. Such research findings can be replicated in other laboratories when they introduce the Barricor tube simply by measuring the within patient variations (SDD) before and after tube introduction.

	Plasma Separator Tube			Barricor Tube				
Troponin range ng/L	Troponin mean, ng/L	trop pairs	y intercept	Troponin mean, ng/L	trop pairs	y intercept	Average reduction in preanalytical error, ng/L	Relative Reduction in preanalytical error, %
100 to 200	170	1500	12.1	147	700	11.1	-4.8	-3.0%
100 to 500	239	4900	36.2	233	1800	33.4	-14.0	-5.9%
100 to 1000	340	7300	104	342	2800	65	-81.2	-23.8%
100 to 2000	524	8700	203	520	3300	116	-166.6	-31.9%

A-252

Targeted Prevalence of Biotin Supplementation in a Melbourne Population of Reproductive Age Women and Its Impact on â-hCG Results

C. M. Trambas[1], K. Liu[1], L. Warren[1], H. Luu[2], C. Tan[1], K. Wollbrandt[1], E. Capovilla[1], B. Leung[1], E. Wu[1], J. Nguyen[1], C. Lynch[1], K. A. Sikaris[1]. [1]*Melbourne Pathology, Collingwood, Victoria, Australia,* [2]*Shimadzu, Rowville, Victoria, Australia*

Background: Changes in clinical practice and consumer behaviour have increased the rate of high dose biotin therapy and supplementation. For laboratories using susceptible immunoassays, this has increased the risk of biotin interference. β-hCG is a test of clinical concern, as a falsely low β-hCG result may obscure pregnancy diagnosis, and permit imaging, procedures or medications that would otherwise be contraindicated. Despite clinical concern, there is no prevalence data on biotin interference affecting β-hCG results. **Objective:** To investigate the prevalence of biotin use and its clinical impact in patients presenting for routine β-hCG testing. **Methods**: Biotin was measured by LC-MS/MS (Shimadzu) in randomly selected serum samples with β-hCG requests over the period May 17 to July 16, 2018. Patients with biotin concentrations > 1 ug/L were followed up to survey biotin supplementation. To model impact on the Roche Elecsys hCG+β assay, we spiked exogenous biotin into samples with varying β-hCG concentrations and evaluated analytical bias. **Results**: 11,900 β-hCG tests were requested over the study period, largely in reproductive age women, and 1564 (13%) were randomised for biotin measurement. Of these, 97% contained biotin below the reference interval of 1 ug/L, and 2.5% contained biotin between 1-5 ug/L. Upon survey, the majority of women with biotin concentrations > 1 ug/L were found to be taking biotin supplements in the 1-3 mg range. Biotin concentrations exceeding the stated tolerance of the Roche assay (30 ug/L) were not detected in any sample. Indeed, our *in vitro* spiking studies suggested significant (> 10%) negative analytical bias only occurred at biotin concentrations of approximately 160 ug/L. The highest concentration of biotin measured in our study (12 ug/L) was more than 10-fold lower than that required to induce such negative bias. **Conclusion**: In a randomly sampled cohort of reproductive age women, 97% of samples contained biotin concentrations considered within normal limits (< 1 ug/L). Whilst 3% of samples contained biotin above this normal range, the majority of these contained < 5 ug/L biotin. Many women with measured biotin exceeding 1 ug/L were taking mg-strength biotin supplements marketed for skin, hair and nail cosmesis. No samples contained biotin concentrations sufficient to cause analytical bias with the Roche Elecsys hCG assay. Together, the data suggest that in our testing population the risk of biotin interference causing a clinically significant, falsely low β-hCG result is extremely low.

A-253

Evaluation of Hemolysis, Lipemia, and Icterus Interference in 19 Assays Performed on the Alinity c System

A. Babalhavaeji[1], V. Kulasingam[2], R. Selvaratnam[2]. [1]*University of Toronto, Toronto, ON, Canada,* [2]*University Health Network, Toronto, ON, Canada*

Background: The Alinity c® clinical chemistry analyser (Abbott Laboratories) has recently been introduced as the next-generation *in vitro* diagnostics system. However, accuracy of results are hampered by presence of hemolysis, lipemia and icterus, as determined in part by thresholds initially defined by the manufacturer. We evaluated the performance of the Alinity c system's 19 assays in the presence of varying degrees of hemolysis, lipemia, and icterus.

Methods: Serum pools were obtained with baseline measurements and spiked with appropriate interferents to prepare samples with varying concentrations of hemolysate, lipemia (using Intralipid® 20% w/v and high endogenous human lipid sample), and conjugated bilirubin. A total of 130 samples were prepared to evaluate 19 analytes. Five separate sample preparations and measurements (n=5) were used to target each level of index for each interferent. The average recovery relative to non-spiked sample was calculated for each level of interference and assessed against the optimal allowable error, defined as 50% of total allowable error, and compared to the manufacturer's package insert claims.

Results: At defined vendor thresholds, most analytes met our allowable error criteria. However, lower thresholds of hemolysis are required for ALT and Creatinine. Phosphate and Creatinine showed interference beyond allowable error with bilirubin concentrations above 68.4 µmol/L and 171 µmol/L, respectively. At triglyceride concentration above 2.3 mmol/L, interference exceeded allowable error for Total Protein. Our optimized thresholds are provided in Table 1.

Conclusion: In lieu of semi-quantitative serum indices, verification by direct assessment of the spectrophotometrically measured interferent concentrations have enabled optimization of thresholds for hemolysis, icterus and lipemia. We observed a general agreement between the manufacturer's claims and our results. However, for ALT, Creatinine, Phosphate and Total Protein, lower thresholds may be warranted for one or more of the three indices.

Table 1. Evaluation and optimization of serum indices for select assays on the Alinity c analyser

	Hemolysis		Icterus		Lipemia		
Assay	Optimized Threshold, g/L (Recommended Index)	Vendor Provided Threshold g/L	Optimized Threshold, µmol/L (Recommended Index)	Vendor Provided Threshold µmol/L	Optimized Threshold, mmol/L (Recommended Index)	Vendor Provided Threshold mmol/L	50% TEa
ALT	1.0 (2+)	7.5	171 (3+)	513	1.7 (3+)	7.1	6.0%
Amylase	2.7 (3+)	1.25	171 (3+)	128	5 (4+)	22.6	6.0%
AST	0.4 (1+)	0.62	171 (3+)	513	None	7.1	6.0%
Total Bilirubin	10 (4+)	20	N/A	N/A	None	8.5	5.0%
CK	None	20	342 (4+)	1026	None	11.3	6.0%
Creatinine	2.7 (3+)	10	171 (3+)	513	None	11.3	4.5%
Glucose 20 (4+) 20 None				712	None	22.6	3.7%
K	1.0 (2+)	1.0	None	712	None	22.6	3.0%
Mg	5.0 (4+)	5.0	342 (4+)	1026	None	11.3	4.0%
Phosphate	1.0 (2+)	1.25	68.4 (2+)	1026	2.26 (4+)	11.3	3.7%
Total Protein	2.0 (3+)	1.25	171 (3+)	513	2.26 (4+)	12.8	3.0 g/L
Uric Acid	None	20	342 (4+)	684	None	22.6	4.5%

For Calcium, CO_2, and Sodium, general agreement was observed across all indices. General agreement was also evident for icterus and lipemia for Albumin, ALP, Chloride and LDH.

A-254

Assessment of the Risk of Biotin Interference Affecting High Sensitivity Troponin T Results in a Melbourne Population

C. M. Trambas[1], K. Liu[1], W. Louey[1], H. Luu[2], K. Wollbrandt[1], C. Tan[1], E. Capovilla[1], B. Leung[1], J. Nguyen[1], E. Wu[1], C. Lynch[1], K. A. Sikaris[1]. *[1]Melbourne Pathology, Collingwood, Victoria, Australia, [2]Shimadzu, Rowville, Victoria, Australia*

Background: The recent FDA warning over a possible falsely negative troponin result in the setting of high dose biotin therapy has reinforced concerns that biotin interference may cause missed diagnosis of acute myocardial infarction (AMI). Biotin exerts concentration dependent negative bias on Roche troponin T results, but whether this translates into a falsely negative result depends on the magnitude of the bias and the true troponin T concentration. At a population level, overall risk is dependent on the prevalence of samples containing high biotin concentrations, as well as the distribution of troponin T results. **Objective**: To determine the prevalence of high biotin concentrations in routine samples presenting for troponin T testing and to evaluate the analytical impact on troponin T results. **Methods**: Biotin was measured by LC-MS/MS (Shimadzu) in consecutive samples presenting for high sensitivity troponin T testing in our laboratory over the period May 17 - August 17, 2018. Troponin T was measured on each sample using the Roche hsTnT Elecsys assay (e801). Biotin-induced analytical bias was characterised by *in vitro* spiking studies, and results were used to model the effects of a given biotin concentration on a given troponin T result. Samples with biotin concentrations 20 ug/L and above underwent troponin I testing on a non-biotin affected assay (Abbott Architect high sensitivity troponin I). **Results**: Our cohort consisted of 3071 samples (51% from women); the median age of patients was 61 (47, 75) and the median eGFR 85 (68, > 90). Approximately 98% of samples contained < 1 ug/L biotin. Just under 2% of samples contained biotin concentrations between 1-10 ug/L, insufficient to cause analytical bias. Eight samples (0.2% total) contained biotin exceeding 20 ug/L, the Roche-declared tolerance of the hsTnT assay. Our spiking studies confirmed that negative analytical bias of 10% and greater occurred at biotin concentrations above 20 ug/L. Yet for each of these samples within our cohort, no significant change in result classification occurred as a consequence of possible biotin-induced negative bias. One sample (from a patient with multiple sclerosis) contained more than 300 ug/L biotin, which is sufficient to cause > 90% negative bias. In this patient, however, Architect high sensitivity troponin I was near undetectable, and any predicted negative bias affecting the Roche hsTnT assay had no clinical consequence. **Conclusions**: 98% of samples presenting to our laboratory for troponin T testing contained biotin concentrations within the normal reference interval of 1 ug/L. Approximately 2% of samples showed biotin concentrations above this level, however the vast majority of these did not contain sufficient biotin to cause analytical bias affecting the Roche hsTnT assay. Whilst 0.2% of the total samples tested contained sufficient biotin to cause >= 10% negative analytical bias, no result re-classification occurred nor was there any associated negative clinical impact. Nonetheless, in the event of an AMI, patients with multiple sclerosis on 300 mg daily biotin are at high risk of a falsely negative result, and mitigation strategies selectively targeting this population are required.

A-255

Therapeutic Monoclonal Antibody as Interference in Patients with Multiple Myeloma-Three Clinical Cases

E. Lellouche, R. Mashiach, G. Bryk, M. Leiva, H. Leskes. *Assuta Ashdod Medical Center, Ashdod, Israel*

Background: For the evaluation and follow up of patients with multiple myeloma (MM), the detection, quantification and typing of monoclonal components (M-spike) by serum protein electrophoresis (SPE) and immunofixation (IFE) methods are critical. In the last few years, new monoclonal antibody (mAb)-based immunotherapies against relapsed/refractory MM were developed. Among them, we can find the anti-CD38 m-Ab Daratumumab. Since Daratumumab is an IgG kappa m-Ab, it can interfere with the interpretation of SPE and IFE results, therefore, it is crucial to identify endogenous M-spikes from Daratumumab one. Hydrashift 2/4 daratumumab is a commercial kit from Sebia designed to overcome this possible interference by forming a complex anti-Daratumumab-Daratumumab and shifting Daratumumab band to the alpha zone in IFE gel. The aim of this study was to assess the interference of Daratumumab with SPE and IFE tests.

Methods: 3 patients who received one intravenous infusion with intervals as follows: 1 week (P1W), two week (P2W) and 1 month (P1M). IgG, IgM, IgA (NV: 700-1600, 40-230 and 70-400 mg/dL, respectively), Kappa/Lambda ratio (K/L) (NV: 0.25-1.65), SPE, IFE and Hydrashift 2/4 were evaluated at baseline, post-Daratumumab infusion and 7d and 14d after administration.

Results: P1W= IgG: 501 and 500; IgM: 26 and 24; IgA: 25 and 23 mg/dL with K/L ratio of 0.07 and 0.06 at baseline and post-Daratumumab were observed respectively. SPE showed one single band in the gamma zone corresponding to two monoclonal IgGK and IgGL (weak) bands in IFE before and after treatment. However, following Hydrashift 2/4 the IgGL diagnosis was confirmed due to the shifting of the IgGK band at baseline and post-Daratumumab. P2W= IgG: 398, 466 and 388; IgM: 8, 9 and 7; IgA: 79, 86 and 74 mg/dL at baseline, post-Daratumumab and 7d respectively with a K/L ratio of 1.32 (baseline) and 1.34 (7d). SPE showed a strong IgGK band post-Daratumumab infusion compared to a weak IgGK band at baseline and after 7 days. Hydrashift 2/4 led to no evidences of any band at any time confirming the results that no monoclonal band was observed in this patient. P1M= IgG: 526, 559, 513 and 517; IgM: 23, 22, 19 and 25; IgA: 26, 25, 23 and 21 mg/dL at baseline, post-Daratumumab and 14 and 30 days respectively. No evidences of any M-spike were observed in SPE. However, IFE showed: IgGL at baseline, IgGK and IgGL post-Daratumumab and again IgGL at 14d and 30d. The use of Hydrashift 2/4 kit ratified monoclonal IgGL at baseline, post-Daratumumab, 14d and 30d. **Conclusion:**It is essential for the laboratory to know the Daratumumab protocol therapy (dosage and administration intervals) to avoid misinterpretations of SPE and IFE results. Based on this study, it is recommendable to draw blood just before the next infusion to decrease the possibility of seeing the therapeutic monoclonal antibody (t-mAbs) in IFE. The use of Hydrashift 2/4 daratumumab kit could help to successfully distinguish Daratumumab from endogenous M-spike, however, we still need to be aware of the possible interference from one of the many other t-mAbs which are used in the clinic.

A-256

Determination of Lipemia Interferences for ALT and AST in Native Lipid-Supplemented Samples

Y. Zheng, R. Pearce, A. McShane. *Cleveland Clinic, Cleveland, OH*

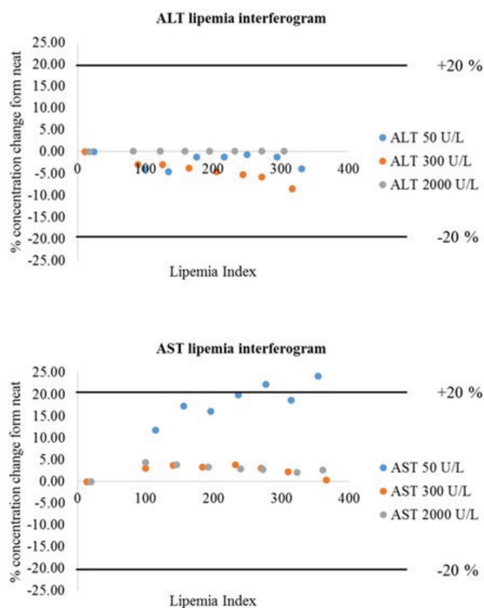
Background: The enzyme activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are biomarkers used in the diagnosis of hepatic disease. In the automated platform, reactions catalyzed by ALT and AST are measured by a coupled reaction which converts NADH to NAD^+ resulting in a decreased signal at 340 nm. Accumulation of lipoproteins (lipemia) in patient samples interferes with the spectrophotometric ALT/AST assay as lipoprotein particles absorbs light strongly at 340 nm. Most of lipemia interference studies have used Intralipid, a commercially available soy-based lipid emulsion to simulate lipemia. However, intralipid consists predominately of phospholipid-rich liposomes and triglycerides-rich chylomicrons, whereas native lipids contain a complex mixture of macromolecular lipid-protein structures. The objective of our study is to determine the lipemia interference of ALT and AST using native lipid-supplemented samples.

Methods: Six plasma pools were made (n>10 each pool) representing enzyme concentrations at 50, 300, and 2000 U/L for ALT and AST. A native lipid pool was collected via ultracentrifugation from patient samples (n>500). The lipemia index levels tested were 100, 150, 200, 250, 300, 350 and 400. Succinctly, the enriched native lipid pool was spiked into each plasma pool to achieve the highest lipemia index level. Subsequently, the combined lipid/patient pool was diluted further with the non-lipemic plasma pool. Experimental design follow CLSI-c56a with triplicate measurements.

Results: By using ±20% from the neat non-lipemic sample as the acceptance criteria, we observed no interferences for ALT activity at 50, 300, and 2000 U/L and AST activity at 300 and 2000 U/L for the range of lipemia index tested here. AST 50 is interference-free up to lipemia index of 200 (Figure 1).

Conclusion: The lipemia interference threshold (150) for ALT and AST may be extended leading to less flagging and manual processing on our automated line.

Figure 1. Lipemia interferograms for ALT and AST using native lipid-supplementation.



A-257

The Impact of Sample Storage and Handling on Functional Complement Assessments using the Binding Site CH50 Assay on the Optilite Analyser

C. Tange, F. Murphy, D. Matters, A. Kay, S. Harding, K. Townsend. *The Binding Site Group Ltd, Birmingham, United Kingdom*

Background: Assessment of functional complement activity using the CH50 assay is an important test identifying both patients with non-specific inflammatory processes (elevated activity) or with classical cascade complement deficiencies (i.e. C1, C2, C3 and C4); the test is also useful in the assessment of patients with systemic lupus erythematosus and other autoimmune conditions. However, it is widely appreciated that appropriate sample storage and handling is required to provide accurate indications of complement activity. Conversely, without a standardised approach to sample storage and handling, complement consumption can occur ex-vivo leading to falsely abnormal results. Here we report on the stability of the classical complement cascade as measured by the CH50 liposome assay developed for use on the Optilite® analyser and comment on the optimal storage methods for reliable result generation.

Methods: Serum was collected from 61 healthy adult donors (self-certification absence of autoimmune conditions, fever or respiratory illness). For each sample, 6 replicates were performed on the day of collection (d0) and thereafter in duplicate with samples stored at either room temperature (approximately 21°C ambient), 4°C or at -20°C (stability was assessed after sequential freeze/thaw cycles). Sample stability was assessed utilising a method based on CLSI guideline EP25-A with measurand drift used as the primary metric with an allowable error of +/-15%. All measurements were determined using the Optilite CH50 assay (The Binding Site Group Ltd, UK).

Results: The median value for samples at d0 was 65.17 U/ml (range 35.17 to 93.82 U/ml), with a median within-sample CV of 0.99% (range 0.18 to 6.2%). After 1d at room temperature there was a median 17% (range -33% to +2%; p<0.001) decrease in CH50 activity. Subsequently there was a persistent decrease in activity in a time dependent manner; allowable error adjusted stability data indicated a median room temperature stability of 1d.

After 1d at 4°C there was a median 9% (range -44% to +3%; p=0.017) decrease in activity. After 2d -11% (range -48% to +8; p=0.006), 3d -13% (range -55% to +4%; p<0.001), 4d -16% (-60% to +6%; p<0.001) and after 7d -21% (-68% to +2% p<0.001). The median % reduction indicated a 4°C stability of 3d; total allowable error adjusted stability data indicated a 4°C stability of 5d.

Samples stored at -20°C following repeat freeze thawing saw a freeze/thaw cycle dependent decrease in CH50 activity. After 1 cycle there was a median 9% (range -22% to +1%; p=0.018) decrease, 2 cycles -11% (range -26% to +2%; p=0.003), 3 cycles -13% (range -38% to +2%; p<0.001), 4 cycles -23% (range -69% to -6%; p<0.001) and after 5 cycles -25% (range -68% to -7%; p<0.001). Allowable error adjusted stability data indicated a maximum of 3 freeze/thaw cycles.

Conclusion: Sample storage and handling can have a significant impact on functional complement assessments. Room temperature storage should only be performed if samples will be analysed on the day of collection, 4°C storage is tolerable providing that assessment is within 3d; freezing samples with limited freeze/thaw analysis would be optimal.

A-258

Solving a Myth: Does Boric Acid Stabilize Aldosterone in Urine at Typical Clinical Laboratory Conditions?

Y. Zheng, R. Pearce, A. Novotny, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Aldosterone is produced by the adrenal glands and plays important role in blood pressure regulation and electrolyte hemostasis. Clinically, measurement of urine aldosterone provides evidence for the diagnosis of hyperaldosteronism and hypoaldosteronism. Urine specimen that is collected in consecutive 24 h is favored specimen type to avoid misdiagnosis because of the large variation in aldosterone secretion. Preservatives such as boric acid are routinely added to the collection containers prior to urine collection. However, little is known if these preservatives can truly stabilize aldosterone in urine. The objective of this study was to determine whether boric acid can stabilize aldosterone in urine at various storage conditions encountered in the clinical labs (ambient, 4 °C and -20 °C).

Methods: Fresh urine samples (within 4 h of collection) were obtained from 10 individuals using sterile containers (mean aldosterone 40.14 ng/dL, range 5.76 to 138.17 ng/dL). Each urine specimen was split into three portions: one without preservative and two with boric acid added at 1 g /100 mL of urine and 0.5 g /100 mL of urine, respectively. Each portion was measured for pH using a pH paper. One aliquot of each specimen was analyzed for aldosterone immediately (time = 0). The remaining samples were then aliquoted and placed at three different storage conditions (ambient, 4 °C and -20 °C). Once the specified storage time was reached, the samples were removed and placed in a -70 °C freezer before analysis. A time = 0 sample was also placed in the -70 °C freezer to test aldosterone stability at -70 °C. All these samples were analyzed on a LIAISON (DiaSorin) analyzer in triplicate. Quality control (QC) data at two levels were collected during the entire study period.

Results: Addition of boric acids did not significantly change measured pH for all 10 individual urine samples. Urine aldosterone were determined to be stable for at least 4 days ambient, 7 days at 4 °C and 90 days at -20 °C (>95 % of data within 2 x CV and 100 % of the data within 3 x CV. CV was calculated from the QC data).The addition of boric at 1g /100 mL of urine and 0.5g /100 mL of urine did not show any significant impact on urine aldosterone stability under the conditions tested.

Conclusion: Aldosterone stability in urine was not affected by addition of boric acid. To ease urine specimen collection and to avoid unnecessary rejection of precious 24 h urine specimens, we have changed the specimen requirement to accept both urine samples with and without boric acid added. Laboratories using other instruments to measure aldosterone should perform an independent study to confirm this finding.

A-259

Evaluation of Clinical Chemistry Laboratory Using Sigma Metrics

T. Cevlik[1], R. Turkal[1], G. Bahramzada[2], O. Unlu[2], A. Tekin[2], O. Sirikci[2], G. Haklar[2]. [1]*Biochemistry Laboratory, Marmara University Pendik Education & Researh Hospital, Istanbul, Turkey,* [2]*Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey*

Background: Six Sigma is a popular quality management system tool, that enables continuous monitoring and improvement of analytical performance in the clinical laboratory. We aimed to calculate sigma metrics and quality goal index (QGI) for 17 biochemical analytes and compared the use of bias from internal quality control (IQC) and external quality assurance (EQA) in the calculation of sigma metrics. **Methods:** This study was conducted in Marmara University Pendik E&R Hospital Biochemistry Laboratory. Sigma metrics calculation was performed as (TEA − Bias)/CV for 17 biochemistry tests analyzed with AU5800 (Beckman Coulter, USA). CV was calculated from IQC data from June 2018 to February 2019 (BC control serum two level/one run per day). EQA bias was calculated as the mean of % deviation from peer group mean, in the last seven surveys and IQC bias was calculated as [(laboratory control result mean - Beckman Coulter control mean)/ Beckman Coulter control mean*100)]. In parameters where sigma metrics was <6; QGI (calculated as QGI = Bias/1.5 CV)

score of < 0.8 indicated imprecision, QGI > 1.2 indicated inaccuracy, and QGI score 0.8-1.2 indicated both imprecision and inaccuracy.

Results: Creatine kinase (both levels IQC), iron (level 2), magnesium (level 2) showed an ideal performance of >6 sigma level for both bias determinations. We obtained same sigma metric and quality goal index ratio results according to both bias for all analytes. **Conclusion:** Sigma metrics is a good quality tool to assess the analytical performance of a clinical chemistry laboratory and it facilitates the comparison of the same assay performance across multiple systems.

Parameter	CV (%)		IQC Bias	Bias (%)	TEA (CLIA)	SIGMA EQC		QGI EQC		SIGMA IQC		QGI IQC	
	Level 1	Level 2				Level 1	Level 2	Level 1	Level 2	Level 1	Level 2	Level 1	Level 2
Albumin	3,48	2,88	0,6	1,78	10	**2,36**	**2,85**	0,34	0,41	**2,70**	3,26	0,11	0,14
ALP	3,87	4,64	3,4	3,67	30	4,14	5,24	0,64	0,81	4,53	5,73	0,39	0,49
ALT	4,45	2,81	2,3	5,88	20	3,17	5,02	0,88	1,40	3,98	6,30	0,34	0,55
Amylase	3,57	2,66	14,1	5,32	30	6,91	9,28	0,99	1,33	4,45	5,98	2,63	3,53
AST	3,75	3,39	4,4	3,82	20	4,31	4,77	0,68	0,75	4,16	4,60	0,78	0,87
CK	3,3	2,4	2,3	4,81	30	7,63	10,50	0,97	1,34	8,39	11,54	0,46	0,64
Iron	3,54	2,7	0,5	3,5	20	4,66	6,11	0,66	0,86	5,51	7,22	0,09	0,12
In. Phosphate	4,94	4,72	1,1	2,75	10	**1,47**	**1,54**	0,37	0,39	**1,80**	**1,89**	0,15	0,16
Glucose	3,64	3,19	1,8	1,2	10	**2,42**	**2,76**	0,22	0,25	**2,25**	**2,57**	0,33	0,38
Calcium	4,68	3,2	1,8	1,34	11	**2,06**	3,02	0,19	0,28	**1,97**	**2,88**	0,26	0,38
Creatinine	4,44	3,65	6,3	5,42	15	**2,16**	**2,62**	0,81	0,99	**1,96**	**2,38**	0,95	1,15
Magnesium	4,24	3,17	0,3	1,97	25	5,43	7,26	0,31	0,41	5,83	7,79	0,05	0,06
T. Bilirubin	4,5	4,24	4,6	2,11	20	3,98	4,22	0,31	0,33	3,42	3,63	0,68	0,72
T. Cholesterol	3,34	3,03	0,6	1,25	10	**2,62**	**2,89**	0,25	0,28	**2,81**	3,10	0,12	0,13
T. Protein	3,8	2,82	0,9	0,68	10	**2,45**	3,30	0,12	0,16	**2,39**	3,23	0,16	0,21
Triglyceride	4,56	4,58	2,3	4,88	25	4,41	4,39	0,71	0,71	4,98	4,96	0,34	0,33
Uric acid	3,05	2,64	1,5	2,3	17	4,82	5,57	0,50	0,58	5,08	5,87	0,33	0,38

A-260

Performance Characteristics of Cardiac CRP High Sensitive Method onARCHITECT *c*8000 Systems

J. Ulloor[1], J. De Giorgio[2], J. King[1], J. Salazar[1], J. Salazar[1], A. Barazzutti[2], L. De Angelis[2], R. Lucini[2], M. A. Beischer[1], K. Bachhawat[1], K. Bachhawat[1]. [1]*Abbott Laboratories, Irving, TX,* [2]*Sentinel Diagnostics, Milan, Italy*

Objective: To assess performance characteristics of the new CRP Vario- Cardiac CRP High Sensitive (cCRP) assay (LN 6K26-31 and 6K26-42) on the ARCHITECT *c*8000 platform.

Relevance: The CRP Vario- Cardiac CRP High Sensitive (cCRP) assay is intended for the quantitation of CRP in serum and plasma. Cardiac CRP high sensitive method may be used for aid in identification and stratification of individuals at risk for cardiovascular diseases. When used in conjunction with traditional clinical laboratory evaluation of acute coronary syndromes, the cCRP may be useful as an independent marker of prognosis for recurrent events, in patients with stable coronary diseases.

Methodology: CRP Vario is a latex immunoassay developed to measure blood CRP levels in serum and plasma. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody, which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change (572nm), with the rate of change being proportional to the quantity of CRP in the sample.

Validation: The specificity of this assay was evaluated against various interferents using a low level and a high level of analyte concentration for each interferent substance. Analyte recovery within ±10% for analyte concentration greater than or equal to 1.00 mg/L or within ± 0.1 mg/L for concentration less than 1.00 mg/L was deemed acceptable. Passing interferent levels for the low level of analyte were found to be 1984.0 mg/dL of Hemoglobin, 500 mg/dL of Intralipid, 62.0 mg/dL of Conjugated bilirubin, 51.0 mg/dL of Unconjugated bilirubin, 2949.0 mg/dL of Triglycerides, 12.0 g/dL of total protein and 243.95 IU/mL of Rheumatoid factor respectively.

Conclusion: The performance of the CRP Vario- Cardiac CRP High Sensitive is comparable to a commercially available method with cardiac claim and can be used for aid in identification and stratification of individuals at risk for cardiovascular diseases.

Performance characteristics of CRP Vario – Cardiac CRP High Sensitive Method

Sample type	Serum and Plasma	
Limit of Quantitation	0.30 mg/L	
Measuring Interval	0.30 to 10.00 mg/L	
Precision	6% or 0.06 mg/L	
Calibration Interval	15 days (360 hours)	
Method Comparison	Cardiac CRP High Sensitive Method vs. Comparative method with a Cardiac Claim	
	N	115
	Slope	1.019
	Y-Intercept	0.061
	Correlation Coefficient	0.996
	Range	0.30- 9.73

A-261

Dried Blood Spots - Applications in Molecular Techniques

J. O. Rodrigues[1], D. Alencar[2], D. Zauli[1]. [1]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil,* [2]*Hermes Pardini Group, Belo Horizonte, Brazil*

Background: In diagnostic medicine, there is increasing interest in using dried blood spot (DBS) cards to extend the range of global health and disease surveillance programs to hard-to-reach populations. DBS is a minimally invasive method and offers a cost-effective solution by simplifying harvesting, preservation, and transport of blood specimens. Furthermore, the use of DBS in diagnostic laboratories has several advantages compared to conventional venipuncture: (i) less blood volume is required, which makes a great difference in harvesting pediatric samples; (ii) the risk of bacterial contamination or hemolysis is minimal; (iii) and DBS can be preserved for long periods with practically no deterioration. **Objective:** to evaluate the performance of DBS samples for 10 molecular genetic tests in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil). **Methodology:** DNA was extracted from EDTA-whole blood and DBS samples from patients of laboratory routine. For DBS, blood samples were potted on FTA cards (Flinders Technology Associates) and FTA cards were dried at room temperature. DNA was isolated from DBS using automated extraction systems and then quantified by fluorometric system. The comparability was performed against DNA isolated from whole-blood samples. Ten different tests were performed using the techniques: Ms-PCR and mTP- PCR (*FMR1*), RFLP-PCR (polymorphism in *MCM6*), ASO-PCR (*HLA, HTT,* and *IT15*), RT-PCR (mutations in the *HFE*), PCR Genotyping (*PAI-1* gene polymorphism), Quantitative Fluorescence PCR (QF-PCR; microdeletions in chromosome Y) and Next Generation Sequence (NGS; *BRCA1* and *BRCA2*). **Results and Discussion:** The DBS samples presented lower DNA concentration when compared to whole blood usually utilized in the tests, but it presented similar performance. Eight tests were validated using DBS samples: Ms-PCR and mTP- PCR (*FMR1*), RFLP-PCR (polymorphism in *MCM6*), ASO-PCR (*HLA, HTT,* and *IT15*), RT-PCR (mutations in the *HFE*), and Next Generation Sequence (NGS; *BRCA1* and *BRCA2*). However, it was not possible to validate the tests for genotyping the *PAI-1* gene and microdeletions on the Y chromosome using the techniques PCR Genotyping and QF-PCR, respectively. **Conclusions and Discussion:** DBS is a simple alternative sample collection procedure that collects much smaller volumes of blood and has simpler storage and transportation conditions compared to other conventional blood, plasma or serum sampling procedures. The preservation of the DNA in the DBS totally depends on the storage conditions, including temperature and humidity, as well as the type of filter paper used. Disadvantages include requirements for assay development and validation as well as the relatively small quantity of DNA isolated from DBS. The results showed that the DNA obtained from DBS extraction using automatic method presented a sufficient quality to be used for PCR and NGS technology.

A-262

Study of Sample Contamination by Carryover in Shared Tube on the Atellica CH and Atellica IM Analyzers

D. Waltrick, C. O. W. Sabino, L. B. Avallone, L. Rodrigues, C. M. M. Dias, E. A. Perobelli, G. A. Campana. *DASA, São Paulo, Brazil*

Background: Total automation systems in the laboratories allowed the use of unified tubes for tests between different analytical systems in flow. However, the induced error in the result of a sample by contamination of the preceding sample is potential. The objective of this study was to evaluate the risk of sample contamination and the clinical impact, when processed in the Atellica CH Analyzer followed by the Atellica IM Analyzer (Siemens Healthineers). **Methods:** TSH, T4, T3, Vitamin D, CEA and ThCG assays was performed according to EP10-A3. The protocol used two samples for each analyte, a sample with normal concentration (CN), divided into nine aliquots, of which three normal interleaved aliquots (CNI) with aliquots of another sample with high concentration (AC), close to the upper limit of the measurement interval, divided into three aliquots. The aliquots were processed in a random assay and a specific sequence in Atellica CH (CN1, CN2, CN3, CA1, CNI4, CA2, CNI5, CA3, CNI6, CN7, CN8 and CN9) and Atellica IM for the trials under study. The acceptance criterion was established considering 3 standard deviations of the CN1, CN2, CN3, CN7, CN8 and CN9 aliquots and compared with the standard deviation of the CNI4, CNI5 and CNI6 interleaved aliquots. **Results**: The results demonstrated that there was no contamination in any of the trials analyzed in the protocol. For all these assays, the standard deviation for aliquots CNI4, CNI5, CNI6, were lower than the acceptable (table below). **Conclusion**: Sample contamination with the preceding sample is statistically insignificant when a serum sample is shared between ATELLICA CH® and ATELLICA IM® systems, due to the efficient probe wash system of ATELLICA CH

that minimizes sample transport to another sample providing the benefit of allowing primary tube sharing with immunodiagnostic systems. **Siemens Healthineers supported the study by providing systems and reagents.*

Table 1. Carryover study data

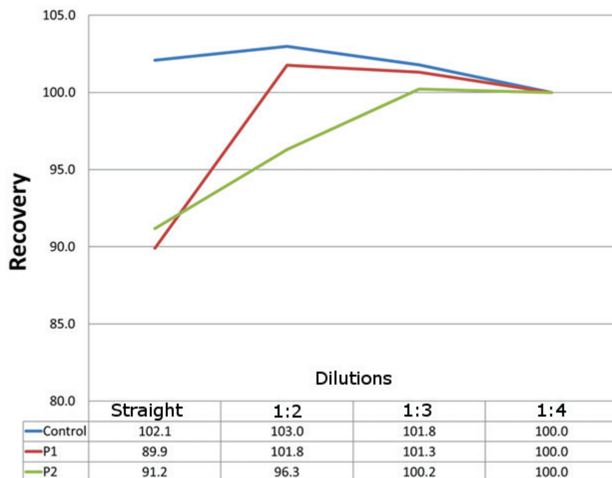
Assay	Units	LL Mean	LL SD	LLI Mean	LLI SD	Carryover	LL 3xSD
T3	ng/mL	0.72	0.02	0.73	0.03	0.9%	0.06
T4	ng/mL	4.67	0.12	4.67	0.15	0.0%	0.36
TSH3UL	µIU/mL	5.78	0.10	5.71	0.06	-1.2%	0.31
ThCG	mIU/mL	7.80	0.37	7.73	0.25	-0.9%	1.12
VITAMIN D	ng/mL	52.09	2.27	49.77	1.85	-4.7%	6.81
CEA	ng/mL	5.28	0.18	5.03	0.09	-5.0%	0.55

A-263

Elimination of Bilirubin Interference in Creatinine Enzymatic Assay by Dilution

A. Charifa, D. R. Bunch, J. M. El-Khoury. *Yale-New Haven Hospital, New Haven, CT*

Background: Hemolysis, icterus and lipemia are the three most common interferences affecting clinical chemistry tests. While lipemia can be removed by ultracentrifugation and in-vitro hemolysis by careful sample re-collection, there is currently no procedure described in the literature to reduce/eliminate the effect of bilirubin. Interference of bilirubin in both Jaffe kinetic and enzymatic creatinine (Cr) assays has been reported in the literature. The falsely lower reported creatinine results can have significant implications on the clinical management of patients. In this study, we investigated if sample dilution can be used to eliminate the effect of bilirubin interference on an enzymatic creatinine assay. **Methods:** We performed an investigative dilution study (straight, 1:2, 1:3 and 1:4) to dilute out the bilirubin interference below the cutoff icterus index (I=15) reported by manufacturer. First, we diluted three control samples with low I index (I<5). A broad range of creatinine concentrations were included (2-5 mg/dL). Afterward, we performed the same dilution study on two samples with an elevated I index P1 (Cr value of 2.05 mg/dl and I index of 52) and P2 (Cr value of 4.45 mg/dl and I index of 40)(figure 1). Average recovery was calculated by averaging the concentrations of samples at the level tested and divided by the average of concentrations tested with icteric index within the acceptable range for creatinine. **Results:** As shown in figure 1, the recovery of the control group with low I index was between 100-103%. And the recovery for the group (P1, P2) with elevated I index showed linear progression and demonstrated resolution of the expected negative interference. **Conclusion:** We demonstrate that dilution of icteric samples is a reliable method for reducing the effects of icteric interference on creatinine measurement and may be applicable to other tests after appropriate validation.



	Straight	1:2	1:3	1:4
Control	102.1	103.0	101.8	100.0
P1	89.9	101.8	101.3	100.0
P2	91.2	96.3	100.2	100.0

A-264

Performance Evaluation of Clinical Chemistry Assays on the Atellica CH 930 Analyzer

P. P. Miranda, C. M. M. Dias, D. P. Aguiar, S. G. R. Santos. *DASA, Recife, Brazil*

Background: The success when introducing a new technology is anchored in good planning. Clinical analysis laboratories have the greatest challenge in planning and correctly conducting the evaluation of new analytical systems, so all efforts must be made with studies that ensure the reliability of the results obtained in patient samples. There is also the challenge of standardizing the equivalence between different analytical systems minimizing impact on clinical interpretation. The main objective of this work is to evaluate performance through an imprecision study of each test individually using the new Atellica CH 930 Clinical Chemistry Analyzers, and the relevance of the identified differences between the three Atellica CH 930 analytical systems. **Methods:** For the imprecision study in the three Atellica CH Clinical Chemistry analyzers, two or three concentrations were used; each level of QC materials was tested in one run per day with five replicates per run for five days, resulting in a total of 25 replicates per sample for Albumin, Calcium, Chlorine, Cholesterol, Creatinine, Magnesium, Potassium, Total protein assays. The imprecision evaluation was performed by the repeatability study (% CVR) and run-to-run variation (% CVWL), according to EP15-A3. For each test, the evaluation criterion was based on the coefficient of variation (CV%) comparison obtained with the manufacturer. In order to determine the performance intersystems the deviation index (Z) was adopted, according of the laboratory goal (Total Error), this index is recommended because it allows a more direct interpretation with the evaluation criterion adopted. **Results:** The %CVR was measured at 1.33%, 0.62% and 0.82 and the %CVWL was 1.66%, 1.48% and 1.16% for Atellica CH 01, Atellica CH 02 and Atellica CH 03, respectively, showing acceptable imprecision, consistent with that reported by the manufacturer. In the evaluation of the intersystems differences it was observed a deviation index against the laboratory target (ET) 0.13, 0.16 and -0.29 for Atellica CH 01, Atellica CH 02 and Atellica CH 03, demonstrating a special deviation laboratory criteria. **Conclusion:** All assays tested on the three Atellica CH 930 analyzers have demonstrated acceptable results with the Siemens Healthineers assays. The deviation indexes are within the required, demonstrating that the equipment presents equivalent analytical performance.

A-265

HbA2 Prime is a Mild Variant but Can Be Misleading

H. F. Mohammad, K. E. Devine, R. M. Young. *LabCorp, Burlington, NC*

Background: Hemoglobinopathies are defined as quantitative and qualitative changes to alpha, and beta globin chains that constitute the adult normal hemoglobin (HbA). The clinical outcome of these blood disorders have been identified as significant and warrants uniform screening for early-on detection and medical intervention before onset of symptoms. Many analytical methods have been developed to detect the more than one thousand hemoglobin variants identified thus far. Currently, we utilize High Performance Liquid Chromatography (HPLC) for screening and high resolution HPLC for confirmation, in addition to the solubility assay for sickle cell screening. Capillary Zone Electrophoresis (CE) method (Sebia Electrophoresis CAPILLARYS 2) has emerged as a very robust separation technique that provides better separation for variants co-eluting with HbA2. This study was conducted to evaluate CE methodology using Sebia CAPILLARYS2 as a complementary method to HPLC.

Methods: Patient samples submitted for hemoglobin fractionation testing have been tested using HPLC (Trinity, Biotech ultra^2) as the primary method and capillary electrophoresis (Sebia, CAPILLARYS2) as the secondary method. Data analysis was performed on the Trinity Biotech Resolution Version 5.3.1, and Sebia phoresis version 9.1.5.

Results: On HPLC, HbA2 prime variants co-elute with HbA which falsely decreased HbA2 level. Decreased HbA2 levels may be interpreted as possible alpha-thalassemia or iron deficiency anemia. The study has shown that capillary electrophoresis can separate HbA2 prime variants from HbA into different electrophoretic zones and thus more accurately determine HbA2 levels.

Conclusion: HbA2 prime is a delta variant that has no known clinical significance. The co-elution of this variant with HbA however, falsely decrease HbA2 level which is indicative of possible quantitative hemoglobinopathies (alpha thalassemia and iron deficiency anemia). This method evaluation study shows that CE can be used to assist and complement HPLC; particularly for the detection of HbA2 prime and accurate determination of HbA2 level.

A-266

Mitigating Biotin Interference in Two Roche Immunoassays by Preincubating Biotin-Conjugated Capturing Molecules with Streptavidin Beads: A Pilot Study

J. Yang[1], J. R. Wiencek[2]. [1]*Medical Laboratories, University of Virginia Health System, Charlottesville, OH,* [2]*Department of Pathology, University of Virginia School of Medicine, Charlottesville, OH*

Background: Modern immunoassays utilize microparticles or beads to immobilize capturing molecules that are used to isolate the immune-complex for signal readout. The capturing molecules are either covalently linked to these materials or non-covalently attached through biotin-streptavidin interaction. Biotin interference on immunoassays utilizing biotin-streptavidin interactions is well recognized; however, not all immunoassays are susceptible. Reagent modifications that incorporate preincubation of biotin-streptavidin materials may offer a solution to immunoassays vulnerable to biotin interference.

Objective: To test whether reagent modification incorporating preincubation of streptavidin-linked beads with biotin-conjugated capturing molecules mitigates biotin interference in originally susceptible Roche sandwich and competitive immunoassays for adrenocorticotropic hormone (ACTH) and antibodies to thyroglobulin (Anti-Tg), respectively

Methods: Roche sandwich and competitive immunoassays utilize three reagent containers for streptavidin beads (M), biotin-conjugated molecule (R1) and ruthenium-labeled antibody (R2). Pools of residual specimens from a single patient were selected to prepare two aliquots with one aliquot spiked with biotin to 1055 ng/mL. Measurements of ACTH and Anti-Tg were analyzed by both the original method and modified reagent described herein. The modified reagent consisted of the same volume ratio of M and R1 reagent. The modified reagent involved spinning down reagent M and saving supernatant (M-Sup). Beads were then incubated with R1 for 30 minutes with constant rotation. This material was spun down and supernatant (R1-sup) was put back into the R1-container. We then re-suspended beads in M-Sup and placed back into the M-container.

Results: Biotin decreased ACTH and increased Anti-Tg measurements with the original reagent from 565 to 32 pg/mL and from 27 to 1652 IU/mL, respectively (Figure). Measurements of ACTH and Anti-Tg with the modified reagent produced equivalent results regardless of biotin interference studied.

Conclusion: The preincubation of streptavidin beads and biotin-conjugated capturing molecules neutralizes biotin interference in the sandwich and competitive immunoassays investigated.

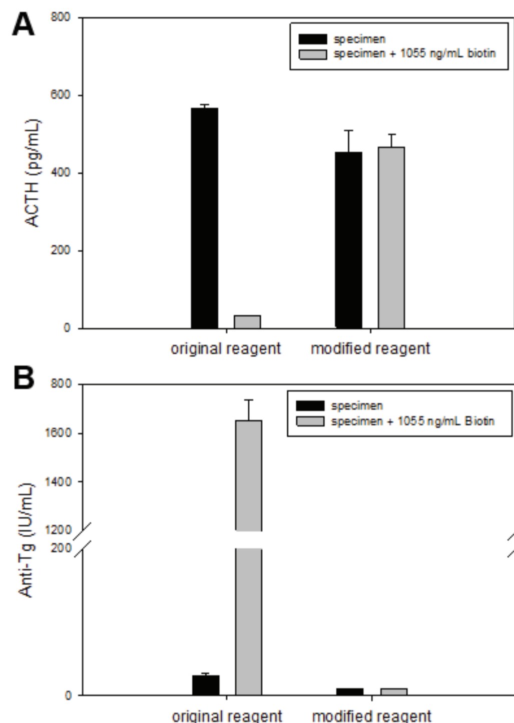


Figure 1. Two aliquots of a patient specimen were created and one aliquot was spiked with 1055 ng/mL biotin. Both aliquots were tested by the original reagent and modified reagent described herein.

A-267

Investigating Bias among POC and Lab INR Methods. Does the INR Adequately Correct for Differences in Reagents?

M. Cervinski[1], L. J. Vaickus[1], C. R. Fantz[2]. [1]*Dartmouth-Hitchcock Medical Center, Lebanon, NH,* [2]*Roche Diagnostics Corporation, Indianapolis, IN*

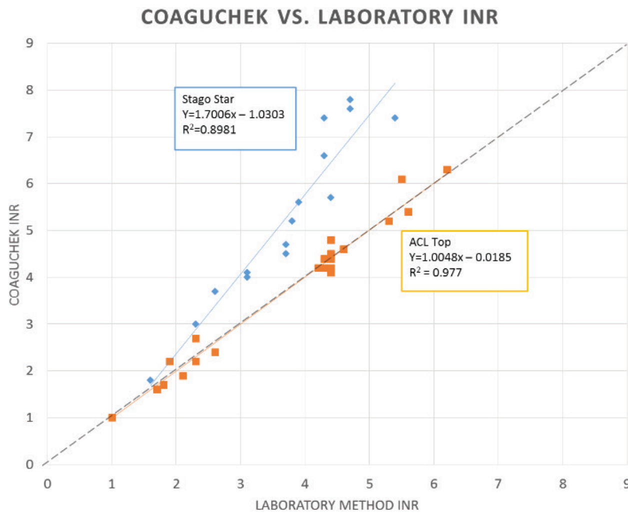
Background: Monitoring of warfarin anticoagulation is frequently accomplished via point-of-care (POC) prothrombin time (PT)/ international normalized ratio (INR) testing. Despite normalization of PT reagents through the use of the INR calculation, inter-method differences exist. Because the highest WHO calibration standard is 4.5, the FDA recommends confirming all POC results greater than this value with a laboratory method. We use the CoaguChek XS meter for POC INR. In the last year we changed from Stago StaR/Satellite to ACL Top 500/300 series analyzers. During this conversion Roche Diagnostics issued a class I product recall for several lot numbers of CoaguCheK XS strips due to reports of patients experiencing high INR values. This notice prompted one of our providers to question the accuracy of a high POC INR that repeated much lower on a Stago laboratory method.

Objective: To compare POC and laboratory INR methods and determine if the observed bias was greater than expected. Secondly, we aimed to identify the appropriate cutoff for laboratory confirmation.

Methods: Paired POC and laboratory INR values collected within six hours of each other from 12/1/2017 to 2/5/2019 were compared via linear regression analysis. Separate analyses were conducted for the CoaguChek vs. Stago and ACL analyzers.

Results: Linear regression analyses of paired CoaguChek and laboratory INR values resulted in equations of CoaguChek=1.7006(Stago)-1.0303, and CoaguChek =1.0048(ACL)-0.0185

Conclusion: Review of the discrepant result revealed the CoaguChek strip used was not of an affected lot and the perceived error between INR methods was within the expected inter-method bias for the CoaguChek and Stago analyzer. Furthermore, a cutoff of 4.5 INR for sending to the lab would not be appropriate for all methods. Laboratories overseeing POC INR programs should be aware of inter-assay



A-268

Field Trial Evaluation of Liquichek Serum Indices Quality Control for Pre-Analytical Monitoring

L. Wong, H. Onishi, M. Gonzales. *Bio-Rad Laboratories, Irvine, CA*

Background: Pre-analytical assessment of patient specimens is arguably one of the most important steps in ensuring quality test results. Specimens compromised by improper handling and the patient condition can interfere with assay performance. Three of the most common specimen interferences that contribute to pre-analytical variation are Hemolysis (H), Icterus (I), and Lipemia (L).

Recently, there has been an emergence of automated instrumentation that has replaced the visual assessment of specimen interferences. The Automated detection of interferences provides several advantages to the laboratory including, increased accuracy and streamlined workflow. As the need for automation increases, so does the need for reliable monitoring of instrument performance. Commercially produced, human-derived sera replicating specimen interferences is now needed to achieve the workflow demands of today's clinical laboratory.

This study evaluates field trial results from four instrument platforms to demonstrate the functionality, commutability, and utility of the third-party Liquichek Serum Indices product.

Methods: Testing was performed in a series of US and European clinical laboratories following the manufacturer's instrument instruction. The product is available in individual 4 mL vials of H, I, L, or non-interfered.Labs pipetted each product into three sample cup and performed single replicate.

Results: Table 1 shows the mean recovery results of Liquichek Serum Indices on four instruments by different manufacturers.

	Roche Cobas	Abbott ARCHITECT	Beckman AU	Siemens Vista
Hemolysis	200	195	+++	4.6
Icterus	20	22	+++	4.4
Lipemia	486	304	++++	4.1
Non-Interfered H / I / L	1 / 0 / 5	1 / 0.4 / -1	N / N / N	1 / 1 / 1

Conclusion: Results demonstrate the functionality, commutability and utility of the third-party Liquichek Serum Indices to monitor an instrument's' ability to detect potential interferences through the HIL pre-analytical test function, thereby increasing the reliability of test results, and ultimately improving patient care.

A-269

Utility of IFCC Standardized LDHI Method in a Pediatric Hospital

J. Pagaduan, E. Tam, S. Devaraj. *Texas Children's Hospital, Houston, TX*

Background: The goal of laboratory test standardization is to achieve equivalent results that are useful for data interpretation and clinical diagnosis. Standardization can be done by calibrating materials to reference standards. Lactate dehydrogenase (LDH) is commonly measured to assess cellular and tissue damage. LDH activity can be measured by conversion of pyruvate to lactate or the reverse reaction. Recently, Ortho Diagnostics made available, the lactate dehydrogenase LDHI Slides technology that is traceable to the IFCC recommended reference method. According to the package insert, LDHI assay could be used for LDH analysis in serum and in plasma and that the assay is insensitive to LDH contained in intact plasma. Furthermore, IFCC and other societies have recommended serum for LDH measurements. Literature reports have shown that platelet contamination results into higher plasma LDH activity. In our pediatric hospital LDH is measured using Vitros Microslide technology and is most often ordered also with other chemistry tests in plasma. Pediatric patients, especially neonates often can only provide limited sample for analysis therefore the aim of this study was to validate the utility of IFCC-standardized Vitros LDHI in serum and plasma samples and compare them to existing LDH assay. **Methods:** LDH was measured in 26 random plasma samples using Vitros LDH slides and LDHI slides. We also evaluated if there is any differences in the LDH measurements based on sample types. LDH from 20 paired serum (collected in red top tubes) and plasma (collected in green lithium heparin with gel separation tubes) samples drawn concurrently were measured using LDHI and LDH slides. Statistical analyses by Deming regression was performed using EP evaluator. **Results:** Our results showed an average of 60% lower LDH activity using LDHI slides compared to LDH slide. Deming regression analysis showed coefficient of correlation, R, of 0.9569, slope of 0.422 and intercept of -11.6. The package insert of LDHI slide indicates that serum or plasma samples could be used for LDHI slides. However, our findings from paired plasma and serum samples revealed that that LDH measurements are on average 38% higher in plasma compared to serum samples. 11 out of 20 pairs had plasma LDH activity greater than 30% compared to serum LDH. Hemolysis is known to increase LDH in the sample and Vitros 5600 reports hemolysis index for each result. The paired samples were drawn concurrently. Comparison of LDH measurements using the LDH slides to LDHI slide using serum revealed better correlation. Deming regression analysis yield R = 0.979, bias of -83.7%, slope of 0.414 and intercept of -2.1. **Conclusion:** Our findings showed that LDH activity measurement using IFCC-traceable Vitros LDHI slides correlated well with Vitros LDH slides in both plasma and serum. However, we observed 38% difference in plasma LDH vs serum LDH in paired samples. Due to the observed differences in plasma and serum LDH activity measurements, sample type could significantly affect the result therefore we recommend that the laboratory restrict the sample type to either plasma or serum for LDH measurements and recalibrate their clinical decision points based on the sample type used.

A-270

Residual Platelets in Heparinized Plasma Lead to Increases in Several Commonly Measured Analytes

L. He[1], K. Dinwiddie[2], L. Bazydlo[1], J. R. Wiencek[1]. [1]*Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA,* [2]*Medical Laboratories, University of Virginia Health System, Charlottesville, VA*

Background: Serum and plasma are two common specimen types used in laboratory testing. Plasma offers the advantage of faster turnaround times, since unlike serum, the blood does not need to clot prior to analysis. Blood tube manufacturers recommend centrifugation settings for efficient separation of cellular material from plasma and of cellular material and clots from serum; however, platelets may not be fully removed under recommended conditions. Inadequate platelet separation may impact measurement of potassium, aspartate aminotransferase (AST), lactate dehydrogenase (LDH) that are present in residual platelets in these specimen types.

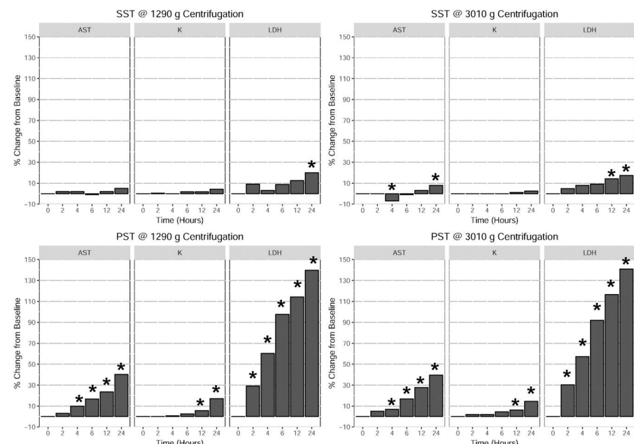
Objective: To determine the effect of inefficient platelet separation on the measurement of several commonly measured analytes in plasma and serum.

Method: Paired blood samples were drawn in lithium heparin plasma separator tubes (PST) and serum separator tubes (SST) from healthy volunteers (n=4). Specimens were centrifuged immediately after draw according to manufacturer recommendations (1290 g for 10 min to remove cellular or clot material or 3010 g for 15 min to reduce platelets). Potassium, AST, LDH were measured on the Abbott Architect c16000, complete blood counts (CBC) were performed on the Sysmex XN-9000, and free hemoglobin (Hb) was measured on the HemoCue Plasma/Low Hb analyzer. After initial measurement, samples were held at 4 degrees Celsius and analyzed at various

time points. Acceptable tolerance limits for analytes were determined by significant change limit (SCL) analysis. For each analyte, SCLs were calculated from 6-months of cumulative performance data (quality control coefficient of variation) multiplied by ±2.8.

Results: Erythrocytes, leukocytes, and plasma/Low Hb were undetectable in all samples and conditions tested. Compared to serum, there was a 20-fold increase in the amount of measurable platelets in plasma regardless of centrifuge settings and more analytes were outside the SCLs (asterisks; $p<0.05$ from t_0).

Conclusion: Residual platelets in plasma are important sources of preanalytical error.



A-271

Assessment about Awareness of Catecholamine Interference in Enzymatic Creatinine Assays among Critical Care Team Members

M. Rathinam[1], K. Relle[1], M. Kim[1], T. Shah[1], A. Muthukumar[2]. *[1]UT Southwestern Medical Center, Dallas, TX, [2]Department of Pathology, UT Southwestern Medical Center, Dallas, TX*

Background: Enzymatic creatinine assays are preferred method for accurate estimation of glomerular filtration rates. Dopamine and dobutamine infusions administered in critical care patients have been shown to cause negative interference in the enzymatic creatinine assays that use peroxidase reaction. Goal of this study is to understand the extent of awareness among the caregivers in critical care areas regarding catecholamine interference in such assays.

Methods: A total 141 caregivers including 53 physicians, 60 nurses and 28 pharmacists from our facility completed the survey. The survey included questions such as preferred site of sample draw in catecholamine infusion patients and method of monitoring renal function, awareness about erratic creatinine results, catecholamine interference in creatinine assays and the specific creatinine method used in their facility. As a part of this study, we also measured creatinine levels using Roche Cobas enzymatic and Jaffe assays in 114 blood samples collected from patients receiving dopamine or dobutamine infusions. This included both PICC/central line and peripheral vein(PV) draw samples.

Results: Among the caregivers, serum creatinine result was the preferred (65%) means for monitoring renal function. Nearly 82% were not aware of dopamine and dobutamine interference in creatinine assay. A similar percentage did not observe erratic creatinine results in their practice. The preferred site of blood collection was equally distributed between PICC and PV draws. More than 90% were not aware of the creatinine method used in their facility. In the patient samples tested, no catecholamine interference was observed with Jaffe or enzymatic creatinine assays in PV draw samples. Less than 10% of PICC line samples had some interference in enzymatic creatinine assay and none observed in PV samples.

Conclusions: Our data suggests dopamine and dobutamine interferes in enzymatic creatinine assays in samples drawn from PICC/central line, but not when drawn from PV. There is lack of awareness about dopamine and dobutamine interference in enzymatic creatinine assays among caregivers in critical care area. It is imperative that core labs play an active role in not only bringing awareness about catecholamine interference in creatinine assays but also providing alternative tests to overcome such interference.

A-272

Extending the Analytical Measuring Range of Turbidometric Homogeneous Immunoassays Using a Novel Kinetic Calibration Method

M. A. Zaydman, J. R. Brestoff, A. M. Gronowski. *Washington University in St Louis, St Louis, MO*

Background: Turbidometric homogeneous immunoassays represent a flexible testing platform that offers short assay time and the potential for full-automation. A major limitation of this assay format is non-linearity in the setting of antigen-excess, i.e. the hook effect. Conventional methods for correcting antigen-excess involve sample dilution and repeated measurement, steps that introduce additional time, costs, and opportunities for laboratory errors. The objective of this study was to develop a novel method for correcting antigen-excess that does not require sample dilution.

Methods: A hybrid computational and experimental approach was employed. A simple mathematical model of a generic turbidometric homogeneous immunoassay was derived and then parameterized by globally fitting to a set of kinetic data from multiple dilutions of a single patient sample with an unusually high concentration of kappa serum free light chains (4408 mg/dL). This model was used to simulate the kinetic behavior of the assay over a wide range of antigen concentrations (10^{-1} to 10^3 mg/dL) and to identify kinetic features that could be used to extend the analytical measuring range. These features were then validated using consecutive clinical samples from two different commonly ordered assays, kappa serum free light chains (n=150) and rheumatoid factors (n=133).

Results: A novel derived kinetic parameter, the Area Under the Curvature (AUCU), was defined as the sum of the finite differences between the normalized kinetic curve and the line connecting the initial and endpoint normalized absorbance changes. The mathematical model predicted that the AUCU would provide a second log-linear calibration curve operating in the zone of antigen excess extending the analytical measuring range by more than 10-fold. As predicted, in both validation datasets, the AUCU demonstrated a log-linear relationship (R^2=0.91 and 0.99 for kappa serum free light chains and rheumatoid factors, respectively) with antigen concentration in the zone of antigen excess.

Conclusions: The barriers to implementing the AUCU method should be minimal as it uses only routinely collected data and does not require significant computing resources or technical expertise. Although additional prospective validation is necessary, the AUCU method has the potential to greatly reduce the burden of sample dilution by allowing quantification of antigen concentration within the zone of antigen-excess.

A-273

Challenges of Blood Spot Heavy Metal Testing - Using Blood Spot Area to Correct for Abnormal Sample Spread when Sample Matrix is Unknown

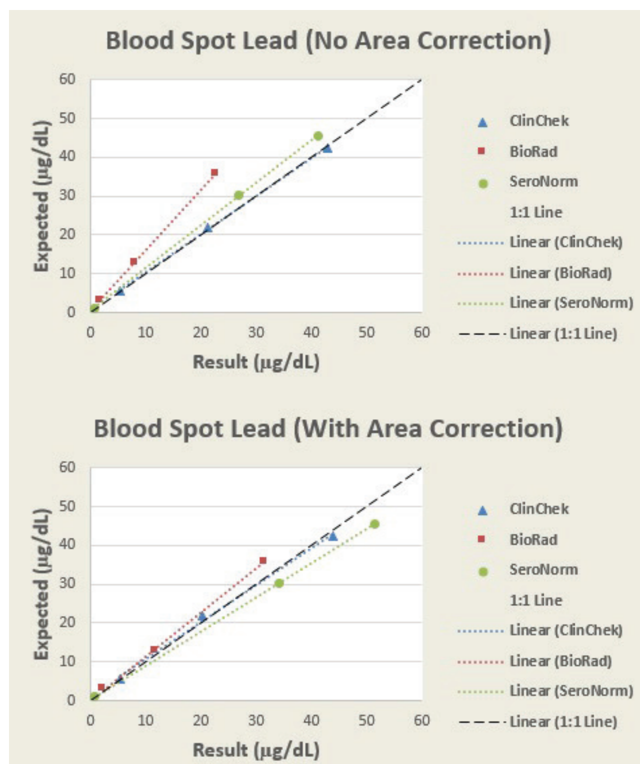
T. Zava. *ZRT Laboratory, Beaverton, OR*

Background: Dried blood spot (DBS) samples consist of whole blood dropped onto laboratory grade filter paper from a finger prick or pipette. It is well known that hematocrit can influence blood spot size, effectively diluting or concentrating the sample if the whole spot is not extracted. In a similar way, whole blood element proficiency samples from agencies such as the Centers for Disease Control and Prevention (CDC) and College of American Pathologists (CAP) with an unknown matrix may spread differently when placed on filter paper than a 100% human blood sample. The purpose of this study is to show that by pipetting a set volume of whole blood, a simple area correction can be applied to account for matrix-related sample dilution or concentration for cadmium, lead, and mercury.

Materials and Methods: The methodology for our DBS element method was presented as a poster at the 2017 AACC conference [Abstract A-402: Dried Urine and Blood Spot Analysis of Essential and Toxic Elements by ICP-DRC-MS]. Whole blood external controls from ClinChek, SeroNorm, and BioRad with known values for cadmium, lead, and mercury were pipetted at 60 µL on filter paper. Blood spot diameter was measured using a caliper to determine area, and two 6-mm punches from each spot were used for analysis. Results were compared before and after area correction. Punches from the inner and outer parts of each blood spot were also tested for homogeneity.

Results: The addition of an area correction significantly improved the accuracy of DBS heavy metal testing when sample matrix is unknown. It was also determined that there was an even spread of analytes across the blood spot.

Conclusions: The use of blood spot area correction for whole blood samples with an unknown matrix pipetted on filter paper can significantly improve the accuracy of results.



Tuesday, August 6, 2019

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

Hematology and Coagulation

A-274

A Pilot Data Analysis of a Metabolomic Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) Based Study of Patients with Sickle Cell/Beta Thalassemia

I. Papassotiriou[1], C. Poziopoulos[1], C. R. Morris[2], E. Voskaridou[3]. *[1]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece, [2]Department of Pediatrics, Division of Pediatric Emergency Medicine, Emory University School of Medicine, Atlanta, GA, [3]Thalassemia and Sickle Cell Disease Center, "Laiko" General Hospital, Athens, Greece*

Background: The complex pathophysiology of Sickle Cell Disease (SCD) makes unlikely that a single therapeutic agent will prevent or reverse all SCD complications. Metabolomic analysis might help in the characterization of the endogenous and exogenous effects of potential new treatments. There are very few reports associated with SCD providing comprehensive measurements of metabolites present in blood. Low arginine bioavailability has been associated with a clinical phenotype of increased hemolytic rate, pulmonary hypertension risk and early mortality. Recently, the FDA approved the use of L-glutamine for the treatment of adults and children with SCD, on the basis of the results of randomized phase 3 clinical trials, while L-arginine's involvement is under investigation[1]. In this context we aimed to quantify targeted metabolites' abnormalities in patients with Sickle Cell/beta thalassemia (HbS/β^{Thal}), to identify pathways that might be of interest to prevent disease complications.

Patients and Methods: Thirty adult Caucasian patients with HbS/β^{Thal} aged 45.6±10.9y, (43% male), at steady-state were enrolled in the study, while 20 age-matched healthy individuals (45% male) served as controls. Along with measurements of hematologic and blood chemistry parameters, targeted metabolome analyses for 13 aminoacids and 2 aminoacid's derivatives were performed after extraction from dry blood spots (DBSs) on filter paper using LC/MS/MS, with derivatization (AB-SCIEX-5500 triple-quadrupole QTRAP®LC/MS/MS-Systems).

Results: Multiple metabolite differences are identified in HbS/β^{Thal} vs. Controls. From metabolites involved in the biosynthesis of glutathione, only L-glutamine's levels were lower in patients with HbS/β^{Thal} compared to controls, while 5-oxyproline levels, a catabolic product of glutathione metabolism, were markedly increased in patients with HbS/β^{Thal} compared to controls. Urea cycle aminoacids, also involved in the production of nitric oxide, L-arginine and L-ornithine concentrations were significantly lower in patients with HbS/β^{Thal} compared to controls, with a trend towards lower L-citrulline in patients with HbS/β^{Thal} p=0.06). Finally, aminoacids involved in catecholamines (dopamine, nor-epinephrine and epinephrine) biosynthesis, such as L-phenylalanine and L-tyrosine and its metabolite succinylacetone levels were significantly lower in patients with HbS/β^{Thal} compared to controls. No significant correlations were found between any metabolites and markers of hemolysis, HbF levels and/or iron burden.

Conclusion: This study confirms prior observations concerning aberrations in multiple blood specific amino acid levels in patients with SCD compared to controls, but this is the first report on Caucasian patients with HbS/β^{Thal} and on whole blood from DBS. We also identified for the first time in Caucasian patients with HbS/β^{Thal} important metabolic abnormalities of glutathione and nitric oxide biosynthesis pathways associated with altered concentrations of the metabolites serving of substrates in these cycles. Importantly, we also demonstrate low levels of L-phenylanine and L-tyrosine, which are essential sources for multiple neurotransmitters biosynthesis and neurobehavioral health. The latter novel observation should be confirmed in larger studies, while measurements of urinary amino acid clearances are necessary to evaluate for potential etiologies of the deficiencies through urinary losses *vs*. low substrate availability, increased utilization or abnormal metabolism. Patients with SCD are in a precarious state with respect to many amino acid deficiencies, all of which may have clinical consequences warranting further investigation. [1]NEJM,2018,19;379:226.

A-275

Predicted Economic Benefits of a Novel Biomarker for Earlier Sepsis Identification and Treatment: A Counterfactual Analysis

C. Paoli[1], M. Reynolds[1], C. Coles[2], M. Gitlin[2], E. Crouser[3]. *[1]Danaher, Brea, CA, [2]BluePath Solutions, Los Angeles, CA, [3]Ohio State University Wexner Medical Center, Columbus, OH*

Objective: To estimate the potential clinical and health economic value of earlier sepsis identification in the emergency department (ED) using a novel diagnostic marker, monocyte distribution width (MDW).

Methods: This was a counterfactual clinical and economic analysis of MDW based on results from a non-interventional, multi-center clinical trial (Crouser et al, submitted to CCM). Data from the pivotal trial were used to estimate the values of three key model inputs: A) actual mean time to antibiotics for standard of care (SOC) sepsis treatment in the ED, B) the estimated proportion of sepsis patients that could have benefited from MDW had the trial been interventional. This was simulated using two factors: 1) a positive MDW test result (>20) and 2) administration of antibiotics after their healthcare provider would have received the MDW test result (which was the actual time stamp of the CBC draw plus 30 minutes turnaround time). Finally, C) the simulated weighted mean time to antibiotics of MDW plus SOC. Using the estimates derived from these methods, the potential clinical benefits on time to antibiotics from acting upon the MDW test result were used as model inputs into a cost consequence analysis. Outcomes such as mortality and length of stay were estimated using time to antibiotic data from Ferrer et al 2014. Costs per day were calculated using the 2015 National Healthcare Cost and Utilization Project data for Diagnosis-Related Groups 870, 871 and 872.

Results: Among the 385 patients with sepsis in our trial, a total of 348 were eligible for inclusion in the model. Ninety-three percent of patients were predicted to benefit based on the timing of MDW results relative to actual treatment with antibiotics and 74% had a positive MDW result resulting in 69% (n=239) which could have potentially benefited and with a reduced time to antibiotics from 4.43 hours using SOC to 2.17 hours using SOC plus MDW. Based on the reduction in time to antibiotics versus SOC, SOC plus MDW may result in a 19.3% reduction (28.1% versus 33.6%) in mortality, a mean reduction of 1.74 days (10.1 versus 11.8) in length of stay, and $4,077 ($23,654 versus $27,731) savings per hospitalization. At the hospital level, based on an established national mean of 206 sepsis hospitalizations per hospital per year, earlier identification with MDW is predicted to result in a total of $839,922 in annual cost savings per hospital.

Conclusions: Improved early identification of sepsis with MDW is estimated to improve both the clinical and economic outcomes of sepsis patients presenting in the ED when compared to the current SOC. Further research is warranted to confirm these model projections.

*CE Marked. Pending clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US.

A-276

Changes in Hematological Indices and Lymphocyte Subsets in Response to Plateletpheresis Donation

A. Borai[1], S. Bahijri[2], M. Mohammadi[1], A. Al Sufyani[1], D. Balgoon[1], S. Boraie[1], A. Al Amri[1]. *[1]King Abdullah International Medical Research Center, King Saud bin Abdulaziz University for Health Sciences, King Abdulaziz Medical City, Jeddah, Saudi Arabia, [2]Department of Clinical Biochemistry, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia*

Background: Plateletpheresis is a technique in which whole blood is collected from a donor followed by platelet (PLT) separation. Plateletpheresis has a significant impact on some biochemical indices after donation. This study aimed to investigate the impact of plateletpheresis on complete blood count (CBC) and lymphocyte subsets over a typical inter-donation interval.

Methods: Healthy male subjects (n = 10) were recruited to study changes in CBC and lymphocyte subsets before and at three intervals following plateletpheresis (1, 8 and 22 days). Repeated measures ANOVA was used to compare quantitative variables between different visits.

Results: Following plateletpheresis, platelet count decreased (-30%) significantly 24 hours after donation (p<0.001) compared to the baseline count with significant repeated ANOVA across different visits (p<0.001, Eta = 0.558). No changes were observed in other variables of CBC. The lymphocyte subsets including CD4, CD8 and CD4/CD8 ratio were decreased at 24 hours after donation (-0.6%, -0.4% and -0.7% respec-

tively) but none was significant. At 24 hours, the proportion of CD19 and CD16-56 were slightly increased 1.6% and 3.3% respectively (p>0.05).

Conclusions: The significant reduction in PLT count after 24 hours of platleltpheresis may have adverse health effect on PLT donors. Plateletpheresis has no significant effect on lymphocyte subsets of the donor.

A-277

Bias in Total Hemoglobin Measurements between ePOC, Rapidpoint 500 and Sysmex XN-10

Z. Mohammed-Ali[1], P. Diker[2], S. Bagherpoor[2], T. Hoang[3], I. Vidovic[2], C. Cursio[2], D. Brinc[4]. *[1]University of Toronto, Toronto, ON, Canada, [2]University Health Network, Toronto, ON, Canada, [3]The Michener Institute for Applied Health Sciences, Toronto, ON, Canada, [4]University of Toronto, University Health Network, Toronto, ON, Canada*

Background: Total hemoglobin is frequently measured by different point-of-care (POCT) devices in a hospital, especially in surgical patients. Accurate and consistent hemoglobin measurements, particularly at the lower range of concentrations, are important, as decisions about blood transfusions are often made at hemoglobin concentrations between 70 and 80 g/L. Our objective was to assess the bias between POCT (epoc and RAPIDPoint 500) and hematology (Sysmex XN-10) hemoglobin measurements.

Methods: Whole blood (n=33-51) total hemoglobin measurements were obtained from the epoc Blood Analysis System, RAPIDPoint 500 System, and Sysmex XN-10. Samples were selected from various hospital units and covered a wide range of concentrations. Total hemoglobin measurements performed by RAPIDPoint 500 and Sysmex XN-10 were also retrieved from the laboratory information system from patients across University Health Network hospitals over a period of nine months (May 2018-Dec 2018). Our criteria for the results retrieved was that the data had to be acquired from specimens from the same patient, collected within 10 min or less of each other.

Results: A positive mean bias of 8.28 g/L was observed with RAPIDPoint 500 compared to Sysmex XN-10. This result was consistent with the positive bias of 8.09 g/L between these two instruments observed in patient samples over the past nine months. There was consistently less than 5% difference between all RAPIDPoint 500 instruments within the hospital, ruling out the possibility that the bias observed relative to Sysmex XN-10 was restricted to a few specific instruments. A bias of 1.84 g/L was observed with epoc compared to Sysmex XN-10.

Conclusion: Hemoglobin measured by RAPIDPoint 500 shows a positive bias compared to the Sysmex XN-10 analyzer. This bias may be of clinical relevance when deciding which patients should receive a blood transfusion.

A-278

Automated and Cost-Efficient Early Detection of Hemolysis in Patients with Extracorporeal Life Support: Use of the Hemolysis-Index of Routine Clinical Chemistry Platforms

M. Bosma, F. Waanders, H. P. Van Schaik, D. Van Loon, S. Rigter, E. Scholten, C. M. Hackeng. *St. Antonius Hospital, Nieuwegein, Netherlands*

Introduction: Extracorporeal life support (ECLS), also known as extracorporeal membrane oxygenation (ECMO), is increasingly used for patients with severely impaired cardiac and/or pulmonary function. Risks associated with ECLS include clot formation, since contact of the blood with the ECLS system results in activation of coagulation. Despite intensive anticoagulation therapy, prevention of clot formation in the system remains a challenge. Clot formation is associated with flow limitation, risk for embolism and complications related to hemolysis, where shear stress and pressure differences in the ECLS system further contribute to. Therefore, monitoring of D-dimers and free hemoglobin (fHb) levels is indicated. Conventional methods for fHb analysis are laborious and not always available. Here we evaluated the suitability of the hemolysis-index (H-index), an internal quality control parameter of clinical chemistry platforms, as a clinical parameter for ECLS patients.

Materials and Methods: The analytical performance of the H-index assay (Roche Diagnostics c502 platform) was evaluated following the Clinical Laboratory Standards Institute guidelines and included within-run and total precision, linearity, level of detection and concordance analyses with the conventional analysis of fHb on a DU800 spectrophotometer. Furthermore, clinical and laboratory data from a cohort of ECLS patients (n=56) was analyzed retrospectively.

Results: The H-index significantly correlated with fHb and showed good analytical performance. The upper reference limit (URL) for the H-index, based on a gamma curve fit of data from control patients (n=4239), was set to 4. Clinically relevant hemolysis was arbitrarily set to 5 times the URL. Hence, an H-index >20 in at least two consecutive blood draws was defined as an H-index peak. This confirmation of elevated H-indices was used to exclude pre-analytical factors causing hemolysis.

During ECLS 19.6% of the patients had an H-index above 20 in at least 2 consecutive blood draws, indicating significant hemolysis. In the patients with clot formation in the pumphead the H-index peaked above 100. Visible clots at other locations did not always coincide with hemolysis. The duration of ECLS and the type of ECLS (VV vs. VA) did not significantly affect H-index elevations. Interestingly, H-index peaks were more prevalent in patients that died during ECLS support (91.0% vs. 57.1% in the total study population, P=0.016).

D-dimer levels were strongly increased (>6000 µg/l) in 60% of the patients with ECLS. Of the patients with visible clots, 80% had strongly elevated D-dimer levels. It should however be noted that in 43% of these patients the D-dimer levels was already strongly increased at the start of ECLS, implying that there was no clear peak in D-dimers. D-dimer levels were not significantly affected by the duration of ECLS, the type of ECLS and were not significantly associated with mortality.

Conclusions: We conclude that the routinely measured H-index is a suitable and cost-efficient alternative for the conventional fHb analysis with good analytic performance. The H-index aids in the early detection of hemolysis in patients with ECLS. A repeated H-index>20 was a predictor of mortality. Marked increases (H-index>100) were seen in patients with thrombi in the pumphead.

A-279

Identification of Iron Status of Anemia Patients by using Red Blood Cell Size Factor (RSF), Microcytic Anemia Factor (MAF) and Low Hemoglobin Density (LHD)

c. ren. *1st affiliated hospital of zhejiang university, hangzhou, China*

Background: Assessing the iron status is important for the management of anemia patients. The aim of this study was to assess the value of Red blood cell Size Factor (RSF), Microcytic Anemia Factor (MAF) and Low Hemoglobin Density (LHD) in the identification of iron status in anemia patients. **Methods:** 301 anemia patients and 51 healthy controls were enrolled. Coulter LH-780 hematology analyzer were used to analyze RSf, MAF and LHD. Additionally, serum ferritin (SF), serum ferritin (SF) and transferrin saturation (TS%) were also analyzed in all patients and controls. **Results:** The RSf was (81.7±9.2) fl and the MAF was (6.4±1.6) in IDA group, which was lower than the control group (RSf 94.5±4.2 fl, MAF 13.1±1.2), ACD group (RSf 95.9±12, MAF 8.3±1.8) and CKD group (RSf 96.2±7.3, MAF 8.1±1.8). In IDA group, LHD was significantly higher than control group (41.6 *vs* 2.1), ACD group (41.6 *vs* 6.7) and CKD group (41.6 *vs* 2.8). Using ROC curve analysis, AUC_{RSf}=0.851, AUC_{MAF}=0.818, $AUC_{LHD\%}$=0.894, AUC_{RDW}=0.844. With $Cutoff_{LHD\%}$ value of 12.1, the sensitivity and specificity of diagnosis of IDA were 89.1%, 89.8%, respectively. In functional iron deficiency group, RSf and MAF were higher than non-functional iron deficiency group and LHD was the opposite(p<0.01). **Conclusion:** In our study, LHD was more valuable than RSf, MAF and RDW in the diagnosis of iron deficiency anemia. It could be a useful marker in identification of iron status in anemia patients.

A-280

Epidemiologist

P. Sandhu[1], L. Nguyen[1], T. Taylor[1], A. Aung[1], J. Gayken[2], M. Marques[3], J. Nichols[4]. *[1]Centers for Disease Control and Prevention, Atlanta, GA, [2]HealthPartners (Retd.), St. Cloud, MN, [3]University of Alabama at Birmingham, Birmingham, AL, [4]Vanderbilt University School of Medicine, Nashville, TN*

Background: Standard laboratory coagulation testing (SLCT) is widely practiced to assess bleeding risk mostly prior to surgery and for patient populations admitted in healthcare settings for other medical conditions. Mostly, SLCT includes prothrombin time (PT), prothrombin/international normalized ratio (INR), partial thromboplastin time (PTT), platelet count, bleeding time (BT)/Clotting time (CT). However, according to existing guidelines, testing should be restricted to patients who are eligible candidates based on their clinical and family history. Published evidence has shown that unnecessary SLCT may lead to harmful consequences such as delayed surgery, unwarranted tests, blood loss, infections, and increased treatment/diagnostic costs. The objective of this study was to investigate the implementation of three laboratory practices for better utilization of coagulation testing: the implementation of clinical guidelines for routine coagulation test ordering, process changes for test ordering panels at the organization level, and education of test ordering staff about the SLCTs

protocols. **Methods:** This systematic review was conducted using the Laboratory Medicine Best Practices (LMBP) Initiative's "A-6" methods, which are reported in detail at the LMBP website. In brief, for each systematic review topic, a systematic review team is formed including LMBP staff and a group of experts representing diverse disciplines, backgrounds, and work settings. The review team conducts a review by (1) developing research question(s) to be answered by the review findings, (2) developing an analytic framework depicting interrelationships among interventions, populations, and outcomes; (3) systematically searching for and retrieving evidence using literature search engines, e.g., PubMed, CINAHL, and EMBASE; (4) collecting relevant data and assessing the quality of evidence; (5) summarizing and translating the evidence of effectiveness into the recommendations; (6) summarizing data about applicability (i.e., the extent to which available effectiveness data might apply to diverse population segments and settings), barriers to implementation, and identified research gaps to be addressed by future research. **Results:** Analysis of 14 relevant studies published through August 2018 from multiple healthcare settings indicated that the implementation of clinical practice guidelines for routine coagulation test ordering produced an overall percent reduction effect of -51.8 (95% CI of -64.1, -44.5, 6 studies) and changes in test panels had an overall percent reduction effect of -59.3 (95% CI of -77.9, -34.7, 8 studies) for inappropriate coagulation testing. Due to the limited available evidence (1 study), no conclusions could be made for the effectiveness of educational practices in order to decrease inappropriate coagulation test ordering. **Conclusions:** Based on available evidence, the implementation of clinical guidelines (at the national and local- level) and interventions including process changes for test ordering panels (e.g. elimination/unbundling of coagulation tests from the automated test order panels and preoperative evaluation panels) are effective in decreasing inappropriate coagulation testing.

A-281

Circulating Long Noncoding RNA STAiR18 to Predict Disease Progression for Patients with Multiple Myeloma

l. zhong, t. jiang, j. zhang, j. chen. *Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, chengdu, China*

Background: In this study, we aimed to find out whether STAiR18 (a newly-found IncRNA, which is closely related to the proliferation of myeloma cells and whose secondary structure is stable) could be a new marker for dynamic monitoring on multiple myeloma (MM) patients, since current R-ISS-stratification criteria for MM patients cannot reflect dynamic tumor-load changes and finding such a new marker is highly needed. **Methods:** After collecting bone marrow, plasma and serum of MM patients, we measured IL-6 level of serum by ELISA, extracted RNA from bone marrow mononuclear cells (BMC), circulated plasma RNA, and analyzed STAiR18 expression by qRT-PCR. In follow-ups of MM patients after chemotherapy, we observed minimal residual disease (MRD) by multi-parameter-flow cytometry (MFC), evaluated changes in circulating STAiR18 expression, and assessed their progression free survival(PFS) and survival time. **Results:** As for the 46 MM patients (28% in R-ISS I, 32% inIIand 38% in III) involved in our study, the STAiR18 expression was 4.97±1.24 times of the 10 cases in control group (P=0.022), and was positively correlated with IL-6 level (r=0.88, P=0.0001). By applying the R-ISS, we found that the STAiR18 expression was increased for R-ISS stage II versus I as well as for R-ISS stage III versus II (2.49 in R-ISS I, 5.47 in II, and 6.93 in III). Initial diagnosis showed significant correlation between the circulating STAiR18 expression and BMC STAiR18 expression (r=0.99,P<0.0001). PFS was 80.0% in R-ISS I, 41.2% in II, and 10.0% in III respectively. Survival was 21.77±1.23 months in R-ISS I, 15.32±1.81months in R-ISSII, and 6.53±1.52 months in R-ISS III. In each group, PFS varied with STAiR18 expression: patients with higher STAiR18 expression than the average value reported worse PFS (mean=3.45, X2=5.49, P=0.019 in R-ISS I; (mean=5.04, X2=15.96, P=6.45E-5 in R-ISSII; mean = 6.45, X2=0.021, P=0.88 in R-ISS III). 11 post-chemotherapy patients achieved complete remission. Follow-up showed constantly-negative MRD for seven patients with constantly low STAiR18 expression, and positive in the other four patients with constantly increasing circulating STAiR18 expression whose turning point in the trend appeared 4-8 weeks earlier before positive results by MFC. **Conclusions:** Circulating STAiR18 has the potential to be a new non-invasive marker for prognostic stratification and dynamic MRD monitoring on MM patients.

A-282

Multicenter Study of the Mid-volume Sysmex CS-2500 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers (Product Availability Varies by Country)

M. Heisig[1], B. Gueney[1], R. Biddle[2], A. Rechner[1], M. Kahl[1], R. Barten[1], A. Wagner[1], I. Birschmann[3], C. Eby[4], J. Francis[5], E. Hod[6], B. Kemkes-Matthes[7], T. Ortel[8], S. Pipe[9]. *[1]Siemens Healthineers, Marburg, Germany, [2]Siemens Healthcare Diagnostics Inc., Newark, DE, [3]Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany, [4]Washington University School of Medicine, St. Louis, MO, [5]Florida Hospital, Orlando, FL, [6]Columbia University, Medical Center, New York, NY, [7]Haemostasis Center, University Hospitals Giessen and Marburg, Giessen, Germany, [8]Duke University, Durham, NC, [9]University of Michigan, Ann Arbor, MI*

Background: The Sysmex® CS-2500 System (CS-2500) was introduced into the US market in 2016 to support routine and speciality testing in mid-volume coagulation laboratories.

Aims: The objective of this study was to compare the performance of two automated coagulation analyzers, the CS-2500 and the Sysmex CA-1500 System (CA-1500), using reagents from Siemens Healthineers. Instrument performance for more than 25 assays including PT seconds & INR with Dade® Innovin® and Thromborel® S reagents, APTT with Dade Actin® FSL reagent, Fibrinogen with Dade Thrombin Reagent, DDimer with INNOVANCE® D-Dimer assay, Coagulation Factors II, V, VII, and X with Dade Innovin reagent, Factor V Leiden with Factor V Leiden Assay, Coagulation Factors VIII, IX, XI and XII with Dade Actin FSL reagent, Lupus Anticoagulant and Thrombin Time with Test Thrombin Reagent was compared. Additional PSI® check results for haemolytic and lipemic influence were investigated.

Methods: Method comparison (MC) studies of the CS-2500 versus the CA-1500 was based on 8954 results (sum over all assays) acquired by testing leftover samples. Precision studies were performed according to appropriate guidelines in three laboratories. The complete dataset contained 41,724 results. Additional performance data were determined for regulatory clearance. IRB approvals were obtained as necessary.

Results: Data correlated well between the CS-2500 and the CA-1500. The MC showed Passing-Bablok regression slopes between 0.86 and 1.08 and Pearson correlation coefficients ≥0.958 (depending on assay). Precision testing for the new device / reagent combinations showed low CV (coefficient of variation) values. The median total precision CV was 3.3%, ranging from 0.7 to 10.5% (depending on assay and sample).

Conclusion: The CS-2500 compares well to the CA-1500 and offers the benefits of improved functionality and ease of use in mid-volume coagulation laboratories. SYSMEX is a trademark of SYSMEX CORPORATION

A-283

Multicenter Study of the High-Volume Sysmex CS-5100 System Compared to the Sysmex CA-1500 System Using Reagents from Siemens Healthineers (Product Availability Varies by Country)

M. Heisig[1], B. Gueney[1], R. Biddle[2], A. Rechner[1], M. Kahl[1], R. Barten[1], A. Wagner[1], I. Birschmann[3], C. Eby[4], J. Francis[5], E. Hod[6], B. Kemkes-Matthes[7], T. Ortel[8], S. Pipe[9]. *[1]Siemens Healthineers, Marburg, Germany, [2]Siemens Healthcare Diagnostics Inc., Newark, DE, [3]Herz- und Diabeteszentrum Nordrhein-Westfalen, Universitätsklinik der Ruhr-Universität Bochum, Institut für Laboratoriums- und Transfusionsmedizin, Bad Oeynhausen, Germany, [4]Washington University School of Medicine, St. Louis, MO, [5]Florida Hospital, Orlando, FL, [6]Columbia University, Medical Center, New York, NY, [7]Haemostasis Center, University Hospitals Giessen and Marburg, Giessen, Germany, [8]Duke University, Durham, NC, [9]University of Michigan, Ann Arbor, MI*

Background: The Sysmex® CS-5100 System (CS-5100) was introduced into the US market in 2016 to support routine and speciality testing in high-volume coagulation laboratories.

Aims: The objective of this study was to compare the performance of two automated coagulation analyzers, the CS-5100 and the Sysmex CA-1500 System (CA-1500), using reagents from Siemens Healthineers. Instrument performance for more than 25 assays including PT seconds & INR with Dade® Innovin® and Thromborel® S reagents, APTT with Dade Actin® FSL reagent, Fibrinogen with Dade Thrombin Reagent, D Dimer with INNOVANCE® D-Dimer assay, Coagulation Factors II, V, VII, and X

with Dade Innovin reagent, Factor V Leiden with Factor V Leiden Assay, Coagulation Factors VIII, IX, XI and XII with Dade Actin FSL reagent, Lupus Anticoagulant and Thrombin Time with Test Thrombin Reagent was compared. Additional PSI® check results for haemolytic and lipemic influence were investigated. **Methods:** Method comparison (MC) studies of the CS-5100 versus the CA-1500 was based on 9087 results (sum over all assays) acquired by testing leftover samples. Precision studies were performed according to appropriate guidelines in three laboratories. The complete dataset contained 41,470 results. Additional performance data were determined for regulatory clearance. IRB approvals were obtained as necessary.

Results: Data correlated well between the CS-5100 and the CA-1500. The MC showed Passing-Bablok regression slopes between 0.87 and 1.12 and Pearson correlation coefficients ≥0.966 (depending on assay). Precision testing for the new device / reagent combinations showed low CV (coefficient of variation) values. The median total precision CV was 3.2%, ranging from 0.7 to 8.8% (depending on assay and sample).

Conclusion: The CS-5100 compares well to the CA-1500 and offers the benefits of improved functionality and ease of use in high-volume coagulation laboratories.

A-285

Evaluation of the Stability of Bivalirudin Effect on Activated Partial Thromboplastin Time (aPTT) in Citrated Whole Blood Samples

J. C. Jara-Aguirre, A. M. Wockenfus, B. R. Kelley, B. M. Katzman, B. S. Karon. *Mayo Clinic, Rochester, MN*

Background: Unfractionated heparin is the most common anticoagulant used during an extracorporeal membrane oxygenation (ECMO). However direct thrombin inhibitors like bivalirudin are being used more frequently due to concerns about heparin resistance, heparin sensitivity, or heparin induced thrombocytopenia. In this setting bivalirudin is monitored using the activated partial thromboplastin time (aPTT) or activated clotting time. Bivalirudin has a short half-life (25 minutes) *in vivo* due to proteolytic metabolism and kidney and liver clearance. Because the drug half-life is short *in vivo*, ECMO providers were concerned that delays in aPTT measurement could affect drug monitoring for bivalirudin. The objective of this study was to determine the effect of bivalirudin on aPTT results after incubation in citrated whole blood for 0.5-4 hours.

Methods: To determine the relationship between bivalirudin concentration and aPTT, residual 3.2% sodium citrate blood samples with a normal aPTT result were spiked with a bivalirudin stock solution (500 ug/mL) (Angiomax), to final concentrations of 0, 0.75, 1.5, 2.0, 5.0 and 7.5 ug/mL. Sodium citrate blood samples were then collected from four healthy volunteers and spiked with bivalirudin to final concentrations of 0.75, 1.5 and 5.0 ug/mL. Samples spiked with varying concentrations of bivalirudin had aPTT measured immediately after mixing; and again after 0.5 h, 1 h, 2 h, 3 h and 4 h of room temperature incubation in citrated whole blood specimens. After centrifugation, aPTT measurements were performed on citrated plasma samples in duplicate using HemosIL/SynthASil reagent and the IL ACL Top 500 coagulation system analyzer (Instrumentation Laboratory). Stability was calculated as percent change in aPTT value compared to sample analyzed immediately after spiking. A change ≥ ±10% was considered a significant bias based upon CLIA allowable error of 15% and accounting for observed precision of the assay.

Results: Addition of 0.75, 1.5, 2.0, 5.0 and 7.5 ug/mL of the bivalirudin prolonged average aPTT result from 29 seconds to 58, 65, 84, 137 and 222 seconds, respectively. Eight blood samples were spiked with 0.75 (2 samples), 1.5 (4 samples) or 5.0 ug/mL (2 samples) bivalirudin to achieve target aPTT values of 58, 65, and 137 seconds. All spiked samples demonstrated <10% change when aPTT was measured after 0.5 h, and 7 of 8 demonstrated <10% change at 1 h incubation compared to baseline (immediately after mixing). In contrast, 3 of 8 samples demonstrated >10% change at 2 h incubation, and 6 of 8 demonstrated ≥10% change at 3 h. In most of cases, aPTT decreased by ≥ 10% after 2 h, but in one sample the aPTT increased after 2 h.

Conclusion: These findings suggest that in patients on bivalirudin, aPTT results are stable in citrated whole blood samples for at least 1 hour after blood collection. After 2 hours or longer in citrated whole blood, aPTT values may begin to change to the extent that bivalirudin monitoring could be affected.

A-286

Eliminating Rejection of Hemolyzed PT and PTTs on Stago Evolution Analyzers

C. Vilk, D. Lee. *MultiCare Health System, Tacoma, WA*

Background: Hemolysis is the rupture or destruction of red blood cells. In vitro hemolysis occurs outside of the body and is most often the result of preanalytical factors such as unsatisfactory phlebotomy attempts, difficulty in locating venous accesses, application of tourniquet for prolonged time, wet-alcohol transfer from the skin into the blood specimen, small or fragile veins, small-gauge needles, vigorous tube mixing and shaking or exposure to excessively hot or cold temperatures. When a hemolyzed sample is received into the laboratory for a prothrombin time (PT) or activated partial thromboplastin time (PTT), the Clinical Laboratory Standards Institute (CLSI) recommends rejecting these samples and have them redrawn. The theory behind rejecting these samples is due to possible clotting factor activation which could lead to erroneous results. Rejecting of these samples causes numerous issues. First, there is a significant delay in the treatment of patients in the emergency room, where a large amount of these samples come from. Discarding and recollecting adds significant work for phlebotomists, laboratories, and other health care providers. Moreover, there is an additional cost associated in a redraw with supplies and personnel.

Methods: Our laboratory performs PT and PTT testing on a Star Evolution from Diagnostic Stago, Inc. This analyzer uses a mechanical end-point detection system for these tests. We analyzed 20 hemolyzed samples for PT and PTT. First, we measured the hemolysis index semi-quantitatively on the Abbott Architect to determine if the amount of hemolysis increased a discrepancy in the results. Next, we ran a PT and PTT on these hemolyzed specimens on the Stago Evolutions. We then compared the hemolyzed results to non-hemolyzed results from the patient redraw.

Results: The results show a slight positive statistical bias between the hemolyzed and non-hemolyzed samples, but little to no clinical differences, regardless of the degree of hemolysis.

Conclusion: The hemolyzed samples sent for routine screening of coagulation testing (PT and PTT) should not be a factor for rejection of these samples because the results show little to no clinical differences. This process saves the patient time (up to an hour or more) and unnecessary needle sticks; along with cost savings for the hospital.

A-287

Association between Anaemia and Chronic Kidney Disease among Pre-Dialysis Diabetes Patients in Kumasi, Ghana

W. K. Owiredu[1], I. W. Ofori-Boadu[1], C. Obirikorang[1], K. O. Boadu[2], E. T. Donkoh[3]. [1]*Kwame Nkrumah University of Science and Technology, Kumasi, Ghana,* [2]*Kumasi South Hospital, Kumasi, Ghana,* [3]*University of Energy and Natural Resources, Sunyani, Ghana*

Background: Anaemia commonly occurs in people with chronic kidney disease, a common complication of diabetes. In diabetics, anaemia may be a sequelae of chronic inflammation and can cause further kidney dysfunction which may contribute to adverse clinical outcomes. Literature on anaemia and/or impact of renal impairment on anaemia in Ghanaian diabetic patients is scant. **Objective/Methods:** The prevalence of anaemia in a cross-section of 217 previously diagnosed diabetes patients with(out) prior evidence of chronic kidney disease (CKD) was compared and associations between haematological parameters and kidney disease examined. Persons 18 to 75 years presenting to the diabetic clinic at Kumasi South Hospital and St. Michael's Hospital, Kumasi with a verifiable diagnosis of type 2 diabetes mellitus were eligible. Patients were excluded if they had received dialysis within the past three months, previously received or currently receiving any form of erythropoietin therapy. Patients who had difficulties understanding the study protocol, and those who failed to give consent were excluded. Additionally, individuals with a clinical history of malaria, cancer, gestational diabetes, type 1 diabetes, previously diagnosed or established kidney disease or had loss of limb due to amputation were also excluded. Blood samples collected under standard conditions were assayed for complete blood profile and renal function investigated using a creatinine-based CKD-EPI equation. Anaemia was diagnosed according to WHO criteria: haemoglobin < 12 g/dL and <13 g/dL for females and males respectively. The stages of CKD were categorized based on the classification system established by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (Levin et al., 2013). Chronic kidney disease (CKD) has been defined as decreased kidney function and/or kidney damage persistent for at least 3 months. Kidney dysfunction is indicated by either serum creatinine level of >1.5 mg/dL or an estimated glomerular filtration rate (eGFR) of less than 60 mL/min/1.73 m^2 according to K/DIGO guidelines for CKD staging. **Results:** The mean age of study participants was 50.7 years (SD= 7.5) ranging from 29 to 67 years. The majority had a history of

hypertension (62.7%). Prevalence of CKD was 15.2%. Median levels of serum creatinine (75.8 vs. 117.2 μmol/L) and urea (3.5 vs 5.6 mmol/L) were significantly higher for participants with CKD than those with normal renal function respectively (p≤ 0.001). Anaemia prevalence was significantly associated with CKD status (p<0.05). A statistically significant linear decline was observed for haemoglobin concentration from stage 1(12.9±1.2) CKD to stage 4 (10.5±0.4) (p≤ 0.001). **Conclusions:** Prevalence of anaemia was 60.4% among patients with CKD compared to 34.1% among those without. There is a strong association between anaemia and CKD for both males and females. The prevalence of anaemia was strongly associated with decline in CKD stage classified by a creatinine-based CKD-EPI equation. Haemoglobin concentration decreased linearly with kidney function. Interventions that improve haemoglobin levels may also be beneficial for pre-dialysis diabetic patients.

References: 1. Levin A, et al.,: **Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group.** *Kidney International Supplements* 2013, **3**(1):1-150. 2. WHO: **Iron deficiency anaemia assessment, prevention, and control: a guide for programme managers**. In. Geneva, Switzerland: World Health Organization; 2012.

A-288

Establishing and Validating Autoverification Rules for Hematology Analysis in Clinical Laboratory

Q. Fu, K. chen, F. Huang, W. Wang. *Zhongshan hospital of Sun Yat-sen University, Zhongshan, China*

Background: Hematology analysis is crucial among in-patients and out-patients in hospital. However, manual verification of hematology analysis is time consuming and tedious, with variation between inter-individual laboratory workers. Meanwhile, the increasing number of specimens, the decreasing number of proficient staff, and clinical demands make autoverification indispensible in the laboratory's future development. The study was to establish and validate a set of autoverification rules for hematology analysis in Department of laboratory medicine, Zhongshan hospital of Sun Yat-sen University.

Methods: Sysmex XN-9000 automated hematology analyzer assembly line was used in the detection of hematology analysis in Department of laboratory medicine, Zhongshan hospital of Sun Yat-sen University. Laboman and LIS were used to construct the algorithm and build the database for autoverification of hematology analysis according to Clinical and Laboratory Standards Institute (CLSI) document Auto 10A and 41 rules of Hematology Review Criteria . The autoverification rules for hematology analysis consisted of quality control, instrument error flags, critical values, the analytical measurement range (AMR), the limit range, and delta check. False positive, false negative and autoverification pass rates were verified and the rules were then adjusted and confirmed according to the verification results.

Results: Agreement was achieved between manual verification by two senior laboratory personnel and verification using the autoverification rules in 99.9% of the cases. The total autoverification passing rate was approximate 81%. False positive and flase negative was 13.6% and 0.01%, respectively. Following implementation of the rules, the laboratory turnaround time (TAT) was reduced by 27.0% (30.9 min versus 42.3 min) and staffing numbers fell from three to two whole time equivalents (WTE). Moreover, after implementing the autoverification rules, the error rate fell to 0.02%, indicating that errors were almost completely eliminated.

Conclusion: Through implementing the autoverification, which accelerated verification and decreased the TAT and the odds of human review errors in the released results, we can save more time and concentrate on verifying the abnormal results and proceeding emergency tests.

A-289

Establishment of a Healthy Donor Reference Range for the T-TAS® 01 PL Chip

J. Dahlen[1], A. H. B. Wu[2], C. Ong[2], K. Bliden[3], M. Southerland[3], J. Fay[3], C. Barbour[3], K. Hosokawa[4], P. Gurbel[3]. [1]*Hikari Dx, Inc., San Diego, CA,* [2]*University of California San Francisco, San Francisco, CA,* [3]*Inova Heart and Vascular Institute, Falls Church, VA,* [4]*Fujimori Kogyo Co., Ltd., Tokyo, Japan*

Background: The T-TAS 01 System with PL chip is a novel, easy to use in vitro diagnostic whole blood measurement system for measuring a patient's overall primary hemostatic ability. The test can be used to identify primary hemostasis defects, by comparing the area under a pressure-time curve (AUC) to a reference range determined from donors with normal primary hemostatic ability. The purpose of this study was to establish a T-TAS 01 PL assay reference range from United States (US) healthy donors and, secondarily, compare the reference range to a previously calculated reference range from Japanese healthy donors.

Methods: Healthy donors were recruited and enrolled according to an IRB-approved protocol at three US locations. Blood samples were collected using a 21 gauge needle into vacuum tubes containing the anticoagulant benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide (BAPA), a thrombin and factor Xa inhibitor. Donors were excluded if they had a history of significant bleeding or primary hemostasis defects, or PFA-100 Col/Epi results > 250 seconds. Blood samples were tested with the T-TAS 01 PL assay between 30 minutes to 6 hours after collection. Statistical analyses were performed using Analyse-It 5.11. Reference ranges were calculated as the central 90% distribution, and the US and Japan donor distribution AUC means were compared using the Student's T-test.

Results: A total of 151 healthy US donors aged 22-70 were included. The reference range for US donors was AUC 263-445 (mean 380), which was not significantly different from the AUC 259-444 reference range (mean 368) for Japanese donors aged 20-45 (p = NS). 90% of the results from the US donors were within the central 90% AUC 259-444 Japanese reference range.

Conclusion: The T-TAS 01 PL chip reference range in healthy US donors was AUC 263-445, which is nearly identical to the reference range established from healthy Japanese donors and confirms the proposed AUC < 260 cutoff for identifying primary hemostasis defects.

A-290

Analytical Performance Characteristics of the T-TAS® 01 PL Chip

J. Dahlen[1], T. Hasegawa[2], S. Ishikawa[2], K. Dobashi[2], K. Hosokawa[2]. [1]*Hikari Dx, Inc., San Diego, CA,* [2]*Fujimori Kogyo Co., Ltd., Tokyo, Japan*

Background: The T-TAS 01 System with PL chip is a novel, easy to use in vitro diagnostic whole blood measurement system for measuring a patient's overall primary hemostatic ability. The test can be used to identify primary hemostasis defects, by comparing the area under a pressure-time curve (AUC) to a reference range determined from donors with normal primary hemostatic ability. The objective of this study was to characterize the analytical performance characteristics of the T-TAS 01 PL assay.

Methods: Aspirin-naïve or aspirin-taking healthy donors were recruited according to an IRB-approved protocol in Japan. Statistical analyses were performed using Analyse-It 5.11. Precision data were collected using blood samples having 3 different PL AUC levels tested in duplicate by 3 operators using 2 instruments and 3 device lots. Sample stability was determined by comparing results from blood tested 30 minutes after collection to blood tested at various times after collection. The effects of heparin, hemodilution, and underfilling blood collection tubes were also evaluated by preparation of contrived samples. Open pouch stability was determined by comparing results from PL chips used immediately after unpouching to unpouched chips incubated at room temperature for various durations.

Results: The results from the precision study showed a within-run CV of less than 15% across the assay range and standard deviation less than 35 AUC units. The sources of error from highest to lowest were within-run > between-operator > between-lot > between-instrument. Blood samples were stable for up to 6 hours at room temperature. There was no significant influence of heparin at therapeutic levels, nor was there an influence of hemodilution up to 20% or 50% filling of the blood collection tube. Unpouched PL chips were stable at room temperature for up to 8 hours before testing.

Conclusion: The T-TAS 01 PL assay demonstrated suitable performance for use as a diagnostic tool to identify primary hemostasis defects.

A-291

The Evaluation of Standardized Sebia's Hydragel 5 vWF Multimers Assay for the Analysis of von Willebrand Factor Multimers

J. Kaplon, D. Meijer, G. J. Baalbergen, W. Van Osch, M. Heckman, A. K. Stroobants. *Academic Medical Center, Amsterdam, Netherlands*

Background: Qualitative defects in von Willebrand Factor (vWF) are being classified based on vWF multimer patterns. The vWF multimers are often analyzed with in-house developed non-standardized assays. Recently a standardized vWFmultimer pattern assay, Sebia's Hydragel 5 vWF multimers, has been developed. In our study this method has been evaluated with the aim of designing an optimal standardized protocol for the analysis of vWF multimers.

Methods: Sixteen patient samples (4 normal, 12 aberrant) were analyzed on Hydrasys 2 Focusing Scan with Sebia's Hydragel 5 vWF multimers kit. We investigated whether the pretreatment of the samples influences the vWF multimer pattern and

whether the adapted protocol can distinguish the aberrant from the normal vWF multimer patterns.

Results: The incubation time, temperature, method of cooling of the sample and the storage conditions between preparation and analysis influenced the vWF multimer pattern. The optimal pretreatment was incubation for 20 minutes at 45 °C in a dedicated heating block, followed by cooling for 2 minutes at 0 °C, after which the sample was applied immediately to the gel. In addition, normalization based on the vWF antigen and the inclusion of a control sample with the normal vWF multimer pattern on the same gel was crucial for the proper classification of the vWF multimer patterns. The spectra of the samples were analyzed with an overlay of the control sample, with the highest peaks of both curves being set at 100%.

Conclusion: Variations in the vWF multimer pattern due to the variation in pretreatment underline the need for a standardized protocol. This adapted protocol of Sebia's Hydragel 5 vWF multimers assay with standardized pretreatment, normalization based on the vWF antigen and interpretation of spectra at 100%, compared to the pattern of a concurrently run normal control, allows a proper differentiation of qualitative defects in vWF.

A-292

Venous, Capillary and Heel-Stick Blood Collection in New MiniCollect® Blood Collection Tubes for Hematological Sample Testing

S. Griebenow[1], M. McLean[2]. *[1]Greiner Bio-One GmbH, Kremsmünster, Austria, [2]Greiner Bio-One North America, Monroe, NC*

Background: Where small sample volumes are critical, for infants, elderly or obese patients, the new MiniCollect (MC) tube allows the highest flexibility and accuracy by collecting blood in unprecedented simplicity. FDA-approved MC K_2EDTA is used to collect, transport, store and evaluate capillary blood specimens for hematology tests.

Methods: Three studies including 459 venous, capillary and heel-stick collections covering relevant medical decision points were done at 3 US-sites using MC tubes compared to BD Microtainer K_2EDTA (MT) tubes. IRB approval and donor informed consent were obtained. Following blood collection, the tubes were inverted 8x and processed according to the IFU. Complete blood counts, including 14 analytes were tested using a DxH800 (BC) and SYSMEX XE-5000, (precision WBC ≤ 3%/RBC ≤ 1.5% specified for both instruments) utilizing the associated reagents. Statistical evaluation was performed using STATISTICA 13.

Results: Evaluation of all clinical data and bias was done on basis of the maximum allowed deviation according to CLIA (Data Innovations). The comparison of MC tubes to MT did not reveal any clinically significant deviations. The bias criteria were met at all medical decision levels for all parameters on both instruments. Slight systematic shifts (EOS, MCHC, and MON) were found when comparing initial values for both tubes due to small variations in reference ranges for venous and capillary blood across data for all sites.

Conclusion: The new MC K_2EDTA is clinically equivalent to the MT K_2EDTA tube. The tube provides an enhanced blood collection device for skin-puncture testing and ensures sample integrity to ensure high quality results for critical venous, capillary and heel-stick sample collections. Availability of a carrier tube (separate or permanently affixed) allows transport for automated analysis.

A-293

Point-of-Care FVIII Test Using an Integrated Microfluidic Consumable with a Fluorogenic Assay

E. Y. Chan[1], R. Yu[1], C. Bae[1], M. Lee[2], Y. Chan[3], J. M. Sommer[4], J. Cheng[1]. *[1]rHEALTH, Cambridge, MA, [2]Biocytogen, Worcester, MA, [3]South Cove Community Health Center, Quincy, MA, [4]JMS Bioanalytical, Alamo, CA*

Background: For patients with hemophilia A, the ability to monitor their FVIII levels is essential to maintaining optimal therapeutic dosing. This decreases the risk for unexpected bleeding and side effects, and increases patient care. There is currently no point-of-care FVIII measurement approach compatible with either fingerprick or venous blood draws. All approaches to FVIII measurements are reliant on skilled clinical laboratory personnel and specialized laboratory equipment for assay automation. While the one stage clotting assay is the most commonly used method for determining FVIII activity, a two-stage chromogenic substrate (CS) assay has been widely used as an alternative method. The CS assay utilizes well defined reagents to measure FVIII dependent proteolytic activity of FXa and does not require a FVIII-deficient substrate plasma to measure coagulation activity. For this reason, the CS assay may be a preferred method for quantifying FVIII levels in a point-of-care device. In this work, we sought to improve the measurement range of the conventional two-stage chromogenic FVIII assay. Additionally, we sought to integrate our assay into a plastic microfluidic consumable capable of performing all the steps of the assay from a fingerprick capillary blood sample with an acceptable time to result.

Methods: We designed, synthesized, and tested various fluorogenic peptide substrates that can be cleaved by FXa. The optimal peptides were tested in a microplate format as well as conjugated to hydrogel microparticles for analysis by microvolume cytometry. The optimized assay was implemented onto a microfluidic device that performed the following steps: whole blood collection (10-20 µL), plasma isolation, mixing with FIXa/FX/$CaCl_2$/phospholipids, and subsequent mixing with the fluorogenic peptide.

Results: Our assay demonstrated a full range of 0.39 - 200% on titration curves of FVIII at a single dilution. This is in contrast to existing chromogenic assays that have two separate dilution ranges, one for high and low calibration ranges. The assay was shown to work in solution and also on functionalized microparticles. We successfully integrated the assay on the consumable. The consumable was tested with spiked whole blood and fingerprick samples, showing a measurement range from 1.24% to 200% with a CV% < 13%. The r^2 was > 0.97 for samples measured via the consumable versus those measured via a commercial microplate-based chromogenic assay. Time-to-result was < 15 minutes from fingerprick to fluorescence readout.

Conclusions: We demonstrated measurement of FVIII from a fingerprick using an integrated consumable that employs a fluorogenic assay. The new fluorogenic two-stage FVIII assay has a broader dynamic range and increased sensitivity over the conventional chromogenic two-stage FVIII assay. The approach has good correlation with a conventional chromogenic FVIII assay. The implementation of a two-stage FVIII assay at the point-of-patient would potentially allow for expansion of the testing methodology to diverse geographic regions, allow for better standardization of FVIII results, increase patient access to therapeutic monitoring, and facilitate pharmacokinetics-driven individualized therapy.

A-294

Performance of the INNOVANCE Heparin Assay for the Quantitative Determination of the Activity of Unfractionated Heparin and Low-Molecular-Weight Heparin in Plasma Using a Single Calibration Curve for Both Heparin Types Employing Four Different Coagulation Analyzers

M. Wilkens, S. Lange, M. Merz, D. Bussfeld. *Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany*

Background: Factor Xa-based chromogenic assays are the preferred method for the quantitative determination of the activity of unfractionated heparin (UFH) and low-molecular-weight heparin (LMWH) in plasma. Originally, calibration of these assays was performed with heparin type-specific calibrators. Over the years, new assays emerged using a single calibration curve for both heparin types. Benefits are reduced risk of calibration curve mix-up in clinical practice, reduced workload, and reduced material complexity.

Aims: Generation and comparison of performance data using the INNOVANCE® Heparin assay kit in combination with a dedicated calibrator set and applications for four different coagulation analyzers (BCS® XP System, Sysmex® CS-5100 System, Sysmex CS-2500 System, and Sysmex CA-660 System) for the quantitative determination of UFH and LMWH, aiming at evaluating validity of concept to use a single calibration curve for both heparin types for all four applications.

Methods: The chromogenic factor Xa-based INNOVANCE Heparin assay kit in combination with a dedicated calibrator set comprising five calibrator levels (traceable to the International Standards for UFH and LMWH) and analyzer-specific applications was investigated. Performance data was generated according to corresponding CLSI guidelines and includes linearity, lower limit of quantitation (LLoQ), precision, interference, and method comparison.

Results: All four applications covered a measuring interval from 0.10 to 1.50 IU/mL as demonstrated by LoQ and linearity studies. When compared to the BCS XP System and analyzed according to Passing Bablok, slopes of 0.99, 0.97, and 0.97 and intercepts of –0.01, –0.01, and –0.04 IU/mL were obtained for the Sysmex CS-5100, CS-2500, and CA-660 Systems, respectively, demonstrating good agreement between the analyzers. Results from precision studies justified performing the assay in single determination for each application (repeatability SD ≤0.030 IU/mL and within-device/lab SD ≤0.038 IU/mL for all applications).

Conclusion: Suitability of the INNOVANCE Heparin assay for heparin activity quantification (UFH and LMWH) using a single calibration curve was demonstrated for all four analyzer applications.

A-295

Performance of the Diazyme Laboratories D-Dimer Assay on the VITROS® 5600 Integrated System and VITROS® 4600 Chemistry System

K. Tobias[1], K. Ackles[2], G. Snodgrass[2], D. M. Borses[1]. [1]*Diazyme Laboratories, Poway, CA,* [2]*Ortho Clinical Diagnostics, Rochester, NY*

The Diazyme Laboratories Inc. D-Dimer assay is used for the determination of fibrinogen/fibrin degradation products (D-Dimer) in human citrated plasma. Measurement of D-Dimer is used as an aid in detecting the presence of intravascular coagulation and fibrinolysis. Thrombus formation is normally followed by an immediate fibrinolytic response. The resultant generation of plasmin causes the release of fibrin degradation products (predominantly containing D-Dimer) into the circulation. Diazyme's D-Dimer Assay is based on a latex enhanced immunoturbidimetric assay. D-Dimer proteins in the sample bind to the specific anti-D-Dimer antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of D-Dimer in the sample. The performance of the Diazyme D-Dimer assay on the VITROS® 5600 Integrated System and VITROS® 4600 Chemistry System was assessed on the VITROS® MicroTip assay processing side of the MicroImmunoassay Center using 8.3 uL of patient sample and the Diazyme D-Dimer reagents. Endpoint absorbance measurements were converted to a concentration using 6-point calibration curve. The accuracy of Diazyme D-Dimer assay was evaluated with 128 serum patient samples 24 of which were altered (0.16 - 7.8 µg/mL FEU) on the VITROS® 5600 System and VITROS® 4600 System then compared to the predicate device Roche Hitachi Modular P following CLSI: EP9-A2 guidelines. The VITROS® 5600 System and VITROS® 4600 System showed excellent correlation with the Roche Hitachi Modular P. VITROS 4600 System R^2 value of 0.9913 with a slope= 1.0338, y-intercept of 0.0782. VITROS 5600 System R^2 value of 0.9921 with a slope= 1.0236, y-intercept of 0.0767.A 20-day precision study conducted on the VITROS® 5600 System and VITROS® 4600 System. The VITROS® 5600 System with D-Dimer sodium citrated plasma samples measuring > 0.5 µg/mL FEU have a within-run CV of 0.7% to 3.6%, a between-run CV of 0% to 1.4%, and a between-day CV of 0.7% to 3.1%. Total imprecision ranges from 1.7 to 4.7% CV . The VITROS® 4600 System D-Dimer sodium citrated plasma samples measuring > 0.5 µg/mL FEU have a within-run CV of 1.3% to 3.9%, a between-run CV of 0% to 2.4%, and a between-day CV of 1.0% to 3.0%. Total imprecision ranges from 2.7 to 4.7% CV. The Limit of Quantification (LoQ) check for the VITROS® 5600 System and VITROS® 4600 System was found to be ≤0.15 µg/mL FEU. At 1.00 and 3.31 µg/mL FEU common interfering endogenous substances of ascorbic acid 176 mg/dL, bilirubin 40.0 mg/dL, conjugated bilirubin 40 mg/dL, hemoglobin 1000 mg/dL ,triglycerides 1000 mg/dL, HAMA 490 ng/mL, Heparin up to 1.5 IU/mL and Rheumatoid Factor 100 IU/ml showed no significant interference (≤ 10%). The Diazyme D Dimer assay run on the VITROS® 5600 System and VITROS® 4600 System demonstrated excellent correlation with the Roche Hitachi Modular P Clinical Chemistry Analyzer, exceptional precision and low-end sensitivity. Additionally, the assay was free from interference by endogenous substances at clinically relevant D-Dimer concentration.

A-296

Use of the Optical Channel Warm to 41°C of the XN-9000 Analyser in Samples with Cold Agglutinins

V. Roccaforte, G. Liuzzi, R. M. Russo, M. L. De Angelis, W. P. Porreca, C. F. Perno, S. Pastori. *ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy*

BACKGROUND: The aim of our study is to compare data obtained from the optical channel warm to 41°C with those obtained by impedance channel preheated for 1 hour in samples that show cold agglutinin.

METHODS: 67 samples with mean cellular hemoglobin concentration (MCHC) values > 36.5 g/dL were tested on the XN-9000 for red blood cell (RBC) counting, hemoglobin and hematocrit. The XN-9000 uses 2 different channel for counting RBC: the optical channel (RBC-O) warm to 41°C and the impedance channel (RBC-I) at room temperature.

RESULTS: The comparison between RBC-I and RBC-O showed a Passing-Bablok regression with y= -0,05 + 1,01x (95% CI of slope 0,96 - 1,06; intercept -0,23 - 0,10). The MCHC compared with MCHC-O showed a regression with y= 4,27 + 0,87x (95% CI of slope 0.75 - 1.04; intercept -1,58 - 8,47). No significant differences are observed between RBC-I and RBC-O (p=0,8656) and between MCHC obtained after warming and MCHC-O (p=0,1588).

CONCLUSIONS: The presence of cold agglutinin in blood samples can be detected by a spurious lowering of RBC count and by a spurious increase of the MCHC. The optical channel warm to 41°C represents a great opportunity to correct RBC count in a rapid way whithout preheating the sample.

A-297

Regression Analysis Techniques to Evaluate the Performance of Several Instruments: A Practical Example with D-Dimers Assay Performed with CS-2500 ®, CA-660® , and Cobas ® c501 Platforms

D. Washburn[1], W. Tang[2], E. Drumm[2], S. Henning[2], K. Blackshear[1], M. Kiger[2], V. M. Genta[2]. [1]*Sentara Healthcare, Norfolk, VA,* [2]*Sentara Virginia Beach General Hospital, Virginia Beach, VA*

Background: To reduce the routine and STAT workload of the chemistry department (cobas® c501), our laboratory decided to investigate the transfer of patient D-Dimers assays to the Hematology CS-2500® and CA-660® platforms. Univariate and multivariable regression analysis techniques were employed to compare patient specimen values as obtained with cobas c501 ®with those as obtained with CS-2500 and CA-660. **Materials:** Instruments: One CS-2500, one CA-660 (Sysmex), one cobas c501 (Roche Diagnostics). Methods: Sixty-three sequential patient specimens were analyzed within 15 minutes with the three platforms. The data were transferred by one of us (E.D) to Minitab® (Version 17, Minitab Inc.) statistical software and analyzed with regression analysis techniques, their residuals diagnostics and graphic representations. **Results:** For the patient values interval 0.2 - 16 mg/L, the relationship between CS-2500, CA-660 (y), and cobas c501 (x) assessed with ordinary least squares (OLS) and orthogonal regression analysis techniques showed very similar regression lines (OLS: $\hat{y}$ = -0.01 + 1.23x; Orthogonal: $\hat{y}$ = 0.04 + 1.19x). Consequently the weighted polynomial least squares regression model was employed to assess this relationship. The WPLR model showed a linear relationship between CA-2500 (y_1), CA-660 (y_2), and cobas c501 instruments (pure error test P=0.2). There were no statistically significant differences between regression lines for CS-2500 and CA-660 (P=0.8). The plots of the standardized residuals showed quasi-normal distribution, no discernible pattern by observation order, three possible outliers (std. del. res.>|3|) and no influential observations (HI< 0.5). The relative differences between the values as obtained with cobas and Sysmex instruments showed that more than 50% of the paired observations had a relative difference exceeding target value ± 20%. Consequently the two platforms could not be used interchangeably. The comparison of the patient values as obtained with CA-660 (y) and those obtained with CS-2500 (x) showed that the regression lines as obtained with the OLS regression model and the orthogonal model showed similar regressions lines (OLS: $\hat{y}$ = 0.05 + 0.99x; Orthogonal: $\hat{y}$ = 0.04 + 0.99x). The weighted least squares regression model showed a linear relationship (pure error test P=0.13), normal distribution of the residuals (Anderson-Darling test P=0.29), no discernible pattern when plotted by the observation order, no outliers (std. del. res.<|3|) and no influential observations (HI<0.5). The plot of the relative differences between CA-660 and CS-2500 showed that 90% of the differences were within the target value and ± 20%. **Conclusions:** The comparisons between D-Dimers values as obtained with cobas c501, CA-660 and CS-2500 platforms using orthogonal and weighted last squares models showed a linear relationship in the interval 0.2 - 16 mg/L. Additionally the CA-660 and CS-2500 platforms showed that 90% of the relative difference between paired assays were within target value ± 20%. Consequently the two instruments could be used interchangeably. The transfer of the assays to the Sysmex platforms resulted in significant operational savings. The availability of statistical software, such as Minitab, was of paramount importance in performing calculation-intense statistical analyses.

A-298

Evaluation of the Abbott Alinity hq Hematology Analyser

A. Kamaludin, H. Mohd Zulkifli, C. K. Sng, T. C. Aw. *Changi General Hospital, Singapore, Singapore*

Background: Abbott Diagnostics has introduced a new hematology analyzer, the Alinity hq. The Alinity is based on Abbott's proprietary multi-angle polarized scatter separation (MAPSS) technology. Abbott has further enhanced the optical evaluation of WBC, RBC and platelets with the use of 8 discrete cellular signals to eliminate overlap of measurement signals for platelets and RBCs of similar size (microcytic RBCs, RBC fragments and large platelets). We evaluated the Alinity's analytical and flagging performance and compared it to our current analyzer (XN9000, Sysmex).

Methods: The performance evaluation included analytical precision (3 levels of controls), correlation with the XN9000 and the Alinity's ability to flag possible ab-

normal cells. As one aspect of the evaluation was to compare the Alinity's flagging performance to our current analyzer, slide review was only performed on samples that were flagged by the XN9000 (n=126). The following flagged criteria were monitored: Blast/Abnormal Lymphocytes (AbnLympho), Atypical Lymphocytes (AtypLympho), Left Shift and Platelet Clumps. Statistical analyses were performed on MedCalc software v18.1 (MedCalc, Ostend, Belgium).

Results: Inter-assay precision showed the following: Hemoglobin (Hb g/dL) were 0.5% at all 3 levels; Red Blood Cells (RBC x106/uL) 0.5-0.8% at all 3 levels; White Blood Cells (WBC x103/uL) 2.2% @Level 1, 1.1% @Level 2 and 0.8% @Level 3; Platelets (Plt x103/uL) 3.5% @Level 1, 1.4% @Level 2 and 1.6% @Level 3. The sigma metrics were: Hb, Plt, and MCV >6.0 at all 3 Levels; RBC >6.0 for Level 1 & 2 and 5.9 for Level 3; WBC 5.2 for Level 1 and >6.0 for Levels 2 & 3. Correlation studies (n=474) between the Alinity hq and XN9000 showed a correlation coefficient of >0.95 for Hb, RBC, WBC, Plt and MCV. Out of the 126 samples that required a slide review on the XN9000, there were 61 (Blast/AbnLympho), 33 (AtypLympho), 28 (Left Shift) and 4 (Platelet Clumps). In these series, Alinity showed a higher accuracy rate compared to XN9000 for the presence/absence of the Blast/AbnLympho flag (83.6% versus 1.6 % respectively) and for Platelet Clumps (100% versus 0%). XN9000 has a higher accuracy rate for Left Shift compared to Alinity (78.6% versus 64.3%). The accuracy rate for AtypLympho flag was comparable for both Alinity and XN9000. The Alinity has a lower rate of false positive flags compared to XN9000 for the following parameters: Blast/AbnLympho flag (14.8% versus 98.4%), AtypLympho (0% versus 42.4%), Left Shift (10.7% versus 21.4%) and Platelet Clumps (0% versus 100%).

Conclusion: At a claimed throughput of 119 tests/hr the Alinity hq is the fastest hematology analyzer based on optical cell counting. The Alinity hematology analyzer is well suited for the laboratory's use. Its high sigma analytical performance, speed and more relevant flagging of possible abnormal cells are attractive features.

A-299

Comparison of the Mid-Volume Sysmex CS-2500 System and the High-volume Sysmex CS-5100 System using Reagents from Siemens Healthineers

A. R. Rechner[1], M. Heisig[1], R. Biddle[2], B. S. Gueney[1], R. Barten[1], M. Kahl[1], A. Wagner[1]. *[1]Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany, [2]Siemens Healthcare Diagnostics Inc., Newark, NY*

Background: The Sysmex® CS-2500 System (CS-2500) as well as the Sysmex CS-5100 System (CS-5100) were introduced into the US market in 2016 to support routine- and speciality testing in mid- and high-volume coagulation laboratories.

Aims: The objective of this study was to compare the performance of two automated coagulation analyzers, the CS-2500 and the CS-5100 using reagents from Siemens Healthineers. Instrument performance for more than 25 assays including PT seconds & INR assays with Dade® Innovin® reagent and Thromborel® S reagent, APTT assay with Dade Actin® FSL reagent, Fibrinogen assay with Dade Thrombin Reagent, DDimer with INNOVANCE® D-Dimer assay, Coagulation Factors II, V, VII, and X assays with Dade Innovin reagent, Factor V Leiden with Factor V Leiden Assay, Coagulation Factors VIII, IX, XI and XII assays with Dade Actin FSL reagent, Lupus Anticoagulant and Thrombin Time assay with Test Thrombin reagent was compared. In addition, a comparison of the PSI® check results for haemolytic & lipemic influence was performed.

Methods: The method comparison (MC) studies were performed testing leftover samples. The MC of the CS-2500 versus the CS-5100 was based on more than 8000 results (sum of results over all assays). Additional performance data were determined for regulatory clearance.

Results: Data correlated very well between the CS-2500 and the CS-5100. The MC showed Passing-Bablok regression slopes between 0.89 and 1.07 and Pearson correlation coefficients ≥0.969 (depending on the assay). The PSI check results for hemolysis & lipemia also correlate very well between the two CS-systems.

Conclusion: Based on these results the two CS-systems are very comparable offering the benefits of improved functionality and ease of use in mid- to high-volume coagulation laboratories.

SYSMEX is a trademark of SYSMEX CORPORATION

A-300

Distinguishing T-Cell and NK-Cell Neoplasms by Flowcytometry

M. Shi, M. Timm, P. Nguyen, D. Jevremovic. *Mayo Clinic, Rochester, MN*

Background:

Normal T-cells express surface CD3 (sCD3), and they are CD2+CD5+CD7+CD16-/+CD56-/+ with T-cell receptor (TCR) gene rearranged; while normal NK-cells lack sCD3 expression, and they are CD2+CD5-CD7+CD16+CD56+ with TCR gene germline. It could be difficult to distinguish T-cell from NK-cell neoplasms when neoplastic T-cells lose sCD3 expression, or neoplastic NK-cells gain CD5 expression. This is particularly the case for a reference flow cytometry laboratory, with no other ancillary test or clinical information available. CD3 is a member of the immunoglobulin superfamily and is composed of CD3ε, δ, γ and ζ chains. The four cytoplasmic CD3 chains form a CD3/TCR complex by associating with TCRα/β or γ/δ chains, and this complex is essential for T-cell development, differentiation and activation. Although cytoplasmic CD3ε and ζ chains are present in NK-cells, there is no CD3/TCR complex. Here, we evaluate a flow cytometric panel containing a monoclonal CD3 antibody (clone SK7, BD Biosciences) specifically binding to the cytoplasmic ε chain of the CD3/TCR complex (refers to cCD3), to determine whether cCD3 could help in distinguishing T-cell from NK-cell neoplasms. **Methods:** Flow cytometry immunophenotyping was performed on 16 patients with sCD3- mature T-cell neoplasms, 11 patients with mature NK-cell neoplasms and 12 normal controls, including 27 peripheral blood, 7 tissue and 5 bone marrow specimens. The T/NK panel to separate T-cell from NK-cell neoplasms included antibodies to CD2, cCD3, CD5, CD7, CD16, CD56 and CD45. In addition, T-cell panel (CD2, sCD3, CD4, CD5, CD7, CD8, CD45, TCR γ/δ) and KIR panel (sCD3, CD8, CD16, CD56, CD57, CD94, CD158a, CD158b, CD158e, NKG2A) were performed. A molecular study for TCR gene rearrangement and an immunohistochemical study for TCRβ (TCRBF1) were performed. **Results:** NK-cells and T-cells from the 12 normal samples showed normal surface antigen expression. As expected, NK-cells from normal controls were negative for cCD3 due to the absence of CD3/TCR complex, whereas T-cells were positive for cCD3. The 16 mature T-cell neoplasms were sCD3- with variable loss of CD2, CD5 or CD7; 13 cases were CD4+, 2 were CD4-CD8- and 1 was CD8+. Although sCD3-, all 16 cases revealed uniform positivity for cCD3, demonstrating T-cell lineage. The T-cell lineage was further confirmed by a clonal TCR gene rearrangement in 10 cases and a positive TCRBF1 immunohistochemical study in 4 cases. The 11 NK-cell neoplasms were CD2+sCD3-CD7+/-CD16+CD56+/-CD94+, 4 of which expressed CD5. Nine of 11 NK-cell neoplasms showed homogenous negativity for cCD3, consistent with NK-cell lineage. Interestingly, 2 NK-cell neoplasms revealed partial cCD3 expression, and the NK-cell lineage in one case was confirmed by germline configuration of TCR gene rearrangement. **Conclusion:** By including CD3 antibody specifically binding to the cytoplasmic ε chain of the CD3/TCR complex (cCD3), flow cytometry provides a reliable and rapid method to help distinguish T-cell from NK-cell neoplasms. Uniform cCD3 positivity and homogenous cCD3 negativity highly suggest T-cell lineage and NK-cell lineage, respectively. Additional confirmatory studies are needed if partial cCD3 expression is encountered.

A-301

Ubiquitin Specific Protease 7 (USP7) Role in Platelet Activation and Formation

K. Wilcots[1], T. McIntyre[2]. *[1]Cleveland State University, Cleveland, OH, [2]Cleveland Clinic Cardiovascular & Metabolic Sciences Department, Cleveland, OH*

Background: Cardiovascular disease (CVD) accounts for 30% of deaths globally. One of the most prevalent underling basis of CVD is thrombosis. Thrombi has been found to contain high amounts of platelets. Our current understanding about thrombosis is primarily focused on the mechanisms that initiate platelet activation; however, the endogenous cellular mechanisms and targets that limit platelet activation have not been completely identified. Anti-platelet drugs are currently used in the clinic and successfully reduced thrombus formation. Current research has shifted towards the investigation of signaling processes that initiate thrombus formation to develop more targeted therapeutics. This work will uncover the importance in USP7 regulation of ubiquitinated substrates in platelets and determine if it can be a therapeutic target or biomarker in thrombotic cardiovascular disease.

Methods: To determine the mechanism by which USP7 contributes to platelet activation and formation proteomics was used to characterize changes in platelet proteins that fluctuated in response to USP7 inhibition. To confirm these changes western blot analysis was used. **Results:** Platelets were pre-incubated with two USP7 inhibitors then activated with collagen. Compared to untreated cells, platelets treated with USP7

inhibitors failed to properly aggregate. In platelets stimulated with collagen, strong staining was observed, indicating platelets could adhere to collagen fibrils compared to cells treated with USP7 inhibitor which clearly showed reduced ability to adhere to the matrix. **Conclusion:** Further studies will elucidate the mechanism by which USP7 modulate platelet formation *in vitro* studies.

A-302

Diagnostic Value of Gastric Fluid Lamellar Body Count in Prediction and Severity of Respiratory Distress

S. Gürcan Kersin[1], O. Unlu[2], H. Bilgen[1], G. Bahramzada[2], T. Cevlik[3], A. Memisoglu[1], H. Ozdemir[1], E. Ozek[1], O. Sirikci[2], G. Haklar[2]. *[1]Department of Neonatalogy Marmara University, Istanbul, Turkey, [2]Department of Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey, [3]Biochemistry Laboratory, Marmara University Pendik Education & Researh Hospital, Istanbul, Turkey*

Background: Respiratory distress syndrome (RDS) occurs due to surfactant deficiency especially in very low birth weight preterms. Transient tachypnea of the newborn (TTN) is a cause of respiratory distress caused by delayed resorption of fetal lung fluid. Lamellar body count (LBC) in gastric aspirates of newborns is being used to identify infants at risk of respiratory distress syndrome (RDS) and transient tachypnea of the newborn (TTN). The aim of this study is to evaluate the diagnostic value of gastric aspirate LBC for the prediction and severity of respiratory distress (RD). **Methods:** A total of 66 infants with a gestational age between 32 and 42 weeks, who were born in January 2019 were enrolled in the study. Gastric fluids were collected within 15 minutes after delivery and samples were kept frozen at -20 ° C in Eppendorf tubes until its use. Dithiothreitol (DTT, Sigma-Aldrich, Germany) was prepared in distilled water (10 mg/mL) and 1 part of gastric aspirate was diluted in 6 parts of DTT for lamellar body count. Counting was performed in the platelet channel of DxH 900 (Beckman Coulter, USA). LBC of babies who developed RD (group 1) were compared with the LBC of babies who did not develop RD (group 2). Subgroup analyses were performed according to the duration of RD (> 2 hours). The results are given as [median x10^3 (IQR)].

Results: The babies (48% girls) included in the study had a mean gestational age of 38.5±1.3 weeks and a mean birth weight of 3242±468 grams. A total of 66 gastric aspirate samples (group 1: 22 and group 2: 44) were analyzed. The cesarean section rate was 54%. Respiratory distress was present in 22% of the babies, and %14 were diagnosed as TTN, while the remaining were diagnosed as delayed postnatal adaptation. None of them had RDS and required surfactant treatment. The comparison of the LBC of group 1; 59.5 (42-70) /μL vs. group 2; 56 (43.75-120.75)/μL showed that LBC was not significantly different (P=0.413) between the groups

Conclusion: We did not find any statistically significant difference between the mean LBC in babies who had RD compared to the group who did not. This result may be due to the higher gestational age of our study group, including moderately and late preterms and term babies who have a lower risk for surfactant deficiency. We also observed that about fifty percent of our babies' RD resolved within 12 hours, indicating a delayed postnatal adaptation.Further studies including larger sample sizes may be necessary to drow a conclusion about the cut-off value for babies with mild to moderate respiratory distress.

A-303

Using Artificial Intelligence (AI) Algorithms to Improve Care Management in Haematology an Example of Middleware Software Use in Daily Practice for Diagnosis of Anemia

J. GALHAUD[1], D. DANGLADE[1], H. WALRYCK[1], P. GONTARD[2], L. STANKEVICH[2]. *[1]LABEXA Group, LE HAILLAN, France, [2]Gontard & Cie Group, MOSCOW, Russian Federation*

Background: CBC count is the main criterion for the diagnosis of anemia. Reticulocyte count allows us to distinguish between various causes of anemia and to monitor bone marrow function and response for a therapy. To be more precise and relevant for anemia exploration, Lab professionals are allowed to add some useful complementary parameters to CBC count. Is it possible to do this automatically in full compliance with medical needs?

Methods: We used GFHC (Groupe Francophone d'Hématologie Cellulaire) recommendations, to add reticulocyte count for CBC in various cases of anemia to give fundamental information to the physician : is it regenerative or not ? Criteria are precise: in adults if any obvious hemorrhagic context is absent, in the presence of normo- or macrocytic anemia (Hb <10 g / dl), either initially or during follow-up with a difference of > 25% from the previous result, it is advisable to first carry out reticulocyte counting. This approach facilitates rapid detection of anemia-hemolytic situations with the subsequent execution of blood smears for hyperreticulocytosis. In addition, to explore macrocytosis (> 105 fl to adult) the search for an etiology justifies the realization of a reticulocyte count and the examination of blood smears. To avoid missing any important cases we have programmed our software with a set of rules to add reticulocyte count for CBC if need be, so to align our daily practice to the GFCH recommendations. See Annex 1.

Results: Since the automatic rules have started, the overall number of reticulocyte have increased by up to 44%. Before this, around 200 reticulocyte counts (average) were added monthly to explore anemia and macrocytosis ; after this, with these new rules, between 500 and 750 reticulocyte counts (average) per month are being added, an increase of twice or more. Both hyperreticulocytosis discovery and early detection of anemia were improved. Also, we have added a scientific explanation for prescribers to the lab report to explain why reticulocytes were added despite them not being prescribed. We received a very positive feedback on how we improved our medical service to better support patients outcome.

Conclusion:

With the systematic use of AI algorithms we improved the medical care of patients, according to GFCH recommendations, for a better diagnosis of anemia. As it avoids human failure, forgetting to apply recommendations, this particular example proves the necessity of AI application in daily laboratory practice.

A-304

IRF and IPF Reference Intervals

F. M. Furtado, A. C. Dias, L. C. M. de Almeida, G. M. Reche, J. A. Silva, K. C. da Silva, A. M. Oliveira, A. L. Barbosa, L. F. A. Nery, R. H. Jacomo, G. B. Barra. *Sabin Medicina Diagnostica, Brasilia, Brazil*

Introduction: Recent advances in hematology analyzers used in clinical laboratories allow the determination of additional hematologic parameters, like immature platelet fraction (IPF) and immature reticulocyte fraction (IRF). They are young cells that may be considered indicators of bone marrow recovery and red blood cell and platelet production. The objective of the present study was to establish reference intervals for IPF and IRF in a Brazilian population.

Methodology: We performed data analysis from routine samples processed at a clinical laboratory. Venous whole blood collected in EDTA were analyzed in a Sysmex XN-10 analyzer (Sysmex Corporation, Japan). All assays were performed within 14 hours of collection and samples were stored at room temperature. Hematologic parameters were evaluated by automated methods on the Sysmex XN-10, according to the manufacturer`s manual. Assays were routinely submitted to internal and external quality controls. To determine IPF reference interval, a nonparametric approach was used; to IRF reference interval, robust approach was used. Tukey's fences was used to identity outliers.

Results: A total of 333 samples with platelet count over 130/mm^3 were evaluated for IPF. Eleven outliers were removed and the remaining 322 samples were included in the data analysis. The median age was 38 years (range 0 - 92 years); 42% were male and 58% female. Median IPF value was 4,6% (range 0.6% - 13.9%). 95% reference interval according to the nonparametric approach was 0,9% to 11.2%. A total of 3193 samples with red blood cell indices within normal range were evaluated for IRF. Two hundred and twenty five outliers were removed and the remaining 2968 samples were included in the data analysis. Median age was 37 years (range 0 - 100 years); 44% were male and 56% female. Median IRF value was 9.6% (range 0.8% - 21.4%). 95% reference interval according to the robust approach was 1.57% to 16.88%.

Conclusion: The reference range obtained for IPF compared well with previous studies with the same series of Sysmex instrument. There was a wider range between our study and another study from a Brazilian center using XE instrument. Sysmex XN-series utilizes different principles for IPF measurement from its previous version, therefore, reference intervals established using XE-series might be invalid for this hematology analyzer. The low reference obtained for IRF was in accordance with a previous Brazilian study. The high reference from our study is higher than that obtained at a previous Brazilian study (16.88% versus 12.1%). This difference may be explained by the use of different instruments, selection criteria, number of samples analyzed (3193 versus 132) or study's patients age (range 13 to 80 years). Our study is limited due to the fact that it did not stratify reference values for age or sex. Even though, most of our results are in accordance with previous studies that tested these stratifications**.** We consider our IPF and IRF reference intervals valid for routine use.

A-305

Implementation of a Fully-Automated Latex Immunoturbidimetric Assay for The Diagnosis of Heparin-Induced Thrombocytopenia

C. C. Pierre, M. Acker, L. Bazydlo. *University of Virginia, Charlottesville, VA*

Background: Heparin-Induced Thrombocytopenia (HIT) is an immune-mediated, adverse reaction to heparin in which heparin binds platelet factor 4 (PF4), triggering the development of heparin-PF4 antibodies (HITAb). HITAbs bind and activate platelets causing thrombosis, platelet consumption, and thrombocytopenia. Diagnosis of HIT consists of a HITAb immunoassay screen with reflex to a serotonin release assay (SRA) for confirmation of positive results. Recently, a fully- automated latex immunoturbidimetric assay (LIA) for detection of HITAbs received FDA clearance. We sought to verify the performance characteristics of the LIA with the aim of implementing the test in a high-volume university hospital laboratory.

Methods: The in-house HITAb LIA was performed on the Instrumentation Laboratory TOP700 analyzer using HemosIL HIT-Ab$_{(PF4-H)}$ reagent. In the LIA, microparticles coated with an antibody that mimics HITAbs agglutinate when bound to PF4 in the reagent mixture. Agglutination is inhibited by HITAbs in the patient plasma that compete for PF4 binding. An inverse relationship exists between absorbance and the concentration of HITAb. The assay is standardized with monoclonal antibody calibrators, with results reported in U/L of HITAb (< 1U/L cut-off for a negative result). The comparator method was a HITAb ELISA performed at a commercial reference laboratory with reflex to SRA for HITAb-positive samples. In brief, the SRA is performed by incubating control platelets with radiolabeled serotonin. Upon addition of heparin and heat-inactivated patient serum, radiolabeled serotonin is released by activated control platelets if a functional HITAb is present in the patient serum. From December 2017 to November 2018, all samples that were sent out for clinical HIT testing at the reference laboratory were aliquoted to run the LIA in parallel. A total of 36 samples were collected. Intra-assay precision was assessed by running manufacturer-provided low and high control samples 10 times in succession, while inter-assay precision was assessed by running low and high samples every day for a period of ten days.

Results: 92% agreement (33/36) was observed between the LIA and ELISA. One of the samples that were discordant between assays tested negative by the ELISA and therefore was not assessed by SRA, while another tested positive by the ELISA, negative by LIA and was confirmed negative by SRA. The final discordant sample tested negative by the LIA but positive by the ELISA and was confirmed positive by SRA. Of the 36 samples tested at the reference laboratory, 12 tested positive for HITAb by ELISA and were reflexed to SRA. Both ELISA and LIA showed 83% agreement (10/12) with the SRA. The coefficient of variance for the intra-assay precision studies was 18% and 5% for the low and high controls respectively. The coefficient of variance for the inter-assay precision studies was 28% and 5% for the low and high controls respectively.

Conclusion: The LIA and ELISA methods compared favorably, allowing for clinical implementation of the LIA. The shortened turn-around time of the LIA will significantly reduce the time to rule out HIT, thereby enhancing patient care. The imprecision on the low control level will require further investigation regarding reagent stability.

A-306

Red Blood Cell Distribution Width and B-Type Natriuretic Peptide in Geriatric Population

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

Background: Red Blood Cell Distribution width (RDW) is measurement given by automated hematology instrument; it measures the heterogeneity of the red blood cells. It is reported as percentage of the standard deviation of the red blood cell volume divided by the mean corpuscular volume. RDW is used to investigate anemia and ineffective erythropoiesis. Several studies have shown correlation between the RDW and adverse cardiovascular outcomes. In addition, it can be used as prognostic marker in congestive heart failure (CHF), myocardial infarction, stroke and pulmonary hypertension.

Methodology: 7390 specimens were collected from patients suspected with cardiac failure residing in Long-Term Care Facilities. All samples were tested for BNP using Centaur XP, Siemens and RDW using Beckman Coulter DxH 800. BNP results were separated based on the New York Heart Association functional class classification. Statistical analysis was done using Analyse-it

Results: 4,442 were female (average age 84 years) and 2948 were male (average age is 81years); 30.2% of the patients had BNP below the reference range, 19.6% were likely to have compensated CHF, 21.8% (BNP >200-400 pg/mL), 28.4% were considered as likely to have moderate to severe CHF (BNP >400 pg/mL). RDW was higher in patients with elevated BNP; the higher the stage NYHA classification the higher RDW. There was no statistical difference between male and female.

NYHA Stage	BNP	RDW >15.2
I	up tp 499	57.8%
II	up tpo 1080	61.0%
III	38->1300	65.7%
IV	147->1300	72.8%

Conclusion: Our results confirmed the notion that RDW correlate with BNP even in geriatric population and can be used as an additional screening tool to assess patients with cardiac failure. However, physician should be aware of the limitation of RDW since it could be elevated for other hematological and inflammatory diseases.

A-307

Multiple Dilutions of Sample in One-Stage Factor VIII Assays are Recommended Even in the Samples Showing Correction of Prolonged Clotting Time in Mixing Study

M. Matsuda[1], M. Emmi[2], Y. Hoshiyama[1], Y. Saijo[3], M. Moriyama[3]. [1]*Niigata University Medical and Dental Hospital, Niigata, Japan,* [2]*Sekisui Medical Co., Ltd., Tokyo, Japan,* [3]*Department of Medical Oncology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan*

Background: The proper sequence of tests performed by clinical laboratories to investigate the cause of prolongation of activated partial thromboplastin time (APTT) has not yet established, to our knowledge. In Japan, first, mixing studies are commonly employed, and, if the mixing studies demonstrate lack of correction of the prolonged APTT, then proceed to confirmatory tests for lupus anticoagulants (LA), whereas if the mixing tests show the correction, the determination of coagulation factor activity, especially factor VIII (FVIII) first, using one-stage clotting assay is performed. In the one-stage FVIII assays, although it is generally recommended to dilute patient plasma with buffer at various ratios to perform the assays according to a parallel-line bioassay (i.e. to detect LA causing falsely decreased FVIII activities), only one dilution ratio is commonly employed in Japan, because the mixing tests precede the assay routinely. However, the routine sequence might not detect LA in the samples with both LA and low levels of FVIII activity when the mixing tests showed the correction, indicating a requirement of employing multiple dilutions of samples in the one-stage FVIII assays, irrespective of the results in mixing tests. The aim of this study is to investigate whether multiple dilutions are required in the one-stage FVIII assay after performing a mixing test, and if required, what dilution ratios are practically recommended in the assay.

Methods: Commercially available normal plasma (PBI, Canada) and anti-phosphatidylserine/prothrombin antibodies (aPS/PT) (MBL, Japan), one of the responsible antibodies for LA activity, were added to the FVIII deficient plasma (Sysmex, Japan) to prepare the samples with FVIII activities of approximately 10, 20, 40 and 80% and/or aPS/PT concentrations of 0, 25, 37.5 and 50 µg/mL. Mixing studies were performed on the samples. The samples were then diluted doubling from 1:5 to 1:80 with buffer, and one-stage FVIII assays were performed on the diluted samples. All tests were performed using APTT-SLA reagent (Sysmex) and Coapresta 3000 analyzer (Sekisui Medical, Japan).The results of mixing tests were evaluated using the index of circulating anticoagulant previously reported, and the results of FVIII assays were evaluated following the parallel-line bioassay.

Results: The mixing studies showed the correction of APTT prolongation in the samples with FVIII activity of 10 and 20%, irrespective of aPS/PT concentrations, and FVII of 40 and 70% with aPS/PT of 25 and 37.5µg/mL. The mixing results demonstrated lack of correction in the samples, containing aPS/PT 50 µg/mL, with FVIII activity of 40 and 70%. The one-stage FVIII assays showed parallelism in the samples without aPS/PT, but non-parallelism in the samples with aPS/PT, representing the falsely decreased FVIII activity due to an aPS/PT. In the samples without aPS/PT, the lower dilution ratio showed reliable FVIII activity. By contrast, the higher dilution ratio showed reliable FVIII values in the samples containing aPS/PT.

Conclusions: The results suggest that multiple dilution is recommended in one-stage FVIII assays, irrespective of the results in mixing tests, and the dilution ratios of 1:5 and 1:40 are practically suited.

A-308

Immature Granulocytes are Efficiently Identified by the Abbott Alinity hq Hematology Analyzer

M. Gandhi, G. Lakos. *Abbott Diagnostics, Santa Clara, CA*

Background: The presence of immature granulocytes (IG) and band neutrophil granulocytes (BN) can be a sign of inflammatory/infectious conditions, but also of malignant diseases, such as myelodysplastic syndrome (MDS), acute promyelocytic leukemia (APL), or chronic myeloid leukemia (CML). Hematology analyzers employ various methods for identifying these abnormalities, including morphological flags and enumeration of IG.The Alinity hq hematology analyzer (Abbott Diagnostics, Santa Clara, CA) reports the absolute concentration and percentage of IG, defined as promyelocytes, myelocytes and metamyelocytes, as part of a 6-part WBC differential. In addition, Alinity hq displays a Left Shift (LS) flag if an increased proportion of BN is detected. **Methods:** The ability of Alinity hq to detect immature neutrophil granulocyte forms was assessed in comparison with manual WBC differential (reference method) on a cohort of 1461 clinical samples from the routine workload of three tertiary health care institutions in The Netherlands, using a Research Use Only software version. The study population included inpatients and outpatients with various ages and conditions, including anemias, hemoglobinopathies, infections, malignant diseases, surgery, newborns, and hematological malignancies. **Results:** Manual and Alinity hq %IG comparison resulted in a correlation coefficient of 0.68 and a Passing-Bablok slope of 1.3 on samples with %IG of > 0.2%, and a WBC count of > 1.00 x 10^9/L. Alinity hq detected the presence of 1% and 2% IG, respectively, with a sensitivity of 72.3% and 67.0% and a specificity of 90.7% and 95.1%. The highest %IG results (up to 27.2%) were reported by Alinity hq in patients with CML and MDS. The sensitivity of the Alinity hq LS flag was 52.6% and 44.7% with a specificity of 91.2% and 88.2%, respectively, when a threshold of 5% or 10% BN was used as sole criteria for left shift. When left shift was defined as the combination of ≥ 5% BN and/or ≥ 1% IG, it was detected with 64.3% sensitivity and 91.1% specificity by the combination of the LS flag and ≥ 1% IG in the automated differential. Samples with a LS flag had significantly higher WBC count (17.8 vs 7.36 x 10^9/L) and neutrophil count (12.23 vs 4.73 x 10^9/L) compared to those without a LS flag. The median (inter-quartile range) of %BN and %IG was 2.76 % (1.00-6.96) vs 0.25% (0.00-1.00) and 0.97% (0.00-3.35) vs 0.00% (0.00-0.25) for samples with and without a LS flag, respectively (p<0.0001 for both).

Conclusion: Alinity hq %IG results and Left Shift flagging demonstrated close correlation and agreement with manual WBC differential results.

A-309

The Coagpia® APTT FS Reagent Demonstrates Optimal Sensitivity to Coagulation Factor Deficiencies

L. Smith[1], S. Harper[2], P. Baker[2], G. Lakos[1]. [1]*Abbott Diagnostics, Santa Clara, CA,* [2]*Oxford Haemophilia and Thrombosis Centre, Oxford, United Kingdom*

Background: The activated partial thromboplastin time (APTT) and prothrombin time (PT) are used to screen patients for coagulation factor deficiency. Sensitivity of the APTT test to detect factor deficiency is reagent composition dependent, and even varies between different lots of the same reagent.

Methods: The sensitivity of the recently launched Coagpia® APTT FS reagent (Sekisui Diagnostics, Lexington, MA, distributed by Abbott Laboratories) to detect intrinsic coagulation factor (XII, XI, IX and VIII) and common coagulation factor (FX, FV and FII) deficiencies was investigated on the CP3000 Coagulation System (Sekisui Diagnostics, Lexington, MA). The study was performed in the laboratory of the Oxford Haemophilia and Thrombosis Centre.

Results: Factor sensitivity curves were generated for the APTT FS reagent by testing pooled normal plasma serially diluted with appropriate factor deficient plasma in duplicates and plotting APTT against the factor activity. Data points were fitted with a power curve, and the goodness of fit was evaluated based on the R2 value. The factor activity that corresponds to the APTT threshold was mathematically determined based on the power curve equation if the R2 value was >0.975 (FVIII, FIX, FXI). In the case of lower R2 values, a point-to-point curve was created, and the factor activity corresponding to the APTT limit was visually assessed on the graph (FII, FV, FX, FXII). The detection threshold of the APTT FS reagent for factor sensitivity were ~35%, 49%, 48% and 43% for FXII, FXI, FIX and FVIII, and ~25%, ~80% and ~40% for FX, FV and FII.

Conclusion: The Coagpia® APTT FS reagent has optimal sensitivity to deficiencies of the intrinsic and common coagulation factors and is well-suited for laboratories performing hemophilia screening.

A-310

Salivary Hepcidin ELISA: NEW and UNIQUE Assay!

M. Herkert, T. Dudek. *DRG Instruments, Marburg, Germany*

Background: Hepcidin is an iron homoeostasis regulator peptide. The bioactive peptide Hepcidin-25 is generated predominantly in the liver. Hepcidin exerts its regulatory function by inhibiting ferroportin, the major cellular iron exporter in the membrane of macrophages, hepatocytes, and the basolateral site of enterocytes.

Methods: The new high sensitive salivary Hepcidin-25 assay is a colorimetric solid phase enzyme-linked immunosorbent assay (ELISA) based on the competitive binding of Hepcidin of the sample and biotinylated Hepcidin to immobilized anti-Hepcidin antibody, followed by the detection with a Streptavidin-HRP conjugate. The total assay time is 1.75 hours.

Results: The Elisa allows the quantification of Hepcidin-25 in saliva covering a measuring range form 68.4 - 8000 pg/mL. The analytical sensitivity of the assay is 68.4 pg/mL, the LoB is 54.2 pg/mL, the LoD is 73.9 pg/mL, and the LoQ is 87.7 pg/mL.

The test shows good reproducibility with mean intra-assay precision of 5.8% and mean inter-assay precision of 10.5%. The mean recovery was determined with 100.9%. Linear dilution gave an overall recovery of 95.3%. We found no matrix interference with up to 4 mg/mL haemoglobin.

Conclusion: Benefits of the new assay are very high sensitivity, straight forward assay procedure with ready-to-use reagents, and a total assay time of 1.75 hours.

Tuesday, August 6, 2019

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

Informatics

A-311

Genome-Wide DNA Methylation Analysis by MethylRAD Reveals the Potential Biomarkers and Mechanism of Colon Cancer

G. zheng, H. Wang, Y. Yang, Y. Zhang. *Qilu Hospital of Shandong University, Jinan, China*

Background: Colon cancer (CC) is characterized by global aberrant DNA methylation that may affect gene expression and genomic stability. Although previous studies have identified methylation changes associated with a limited set of genes, profiles of DNA methylation of CC still has not been elucidated clearly. The present study aims to characterize the profile of DNA methylation of CC systemically, and acquire potential bimomarkers and genes regulated by aberrant methylation.

Methods: The genome-wide DNA methylation profile of CC was constructed using MethylRAD technology which can discriminates between CG and non-CG methylation sites. The differential methylated sites (DMSs) and deferentially methylated genes (DMGs) were identified by EdgeR using R software. Then DMGs were subjected to Gene Ontology (GO) and KEGG pathway enrichment analysis. Meanwhile, the RNA expression data of DMGs which could be mapped in Ensemble v70 were downloaded and analyzed from TCGA database. Then the intersection analysis was performed between gene methylation level and RNA expression level. The intersectional genes was subjected to determine the significant canonical pathways and gene networks involved in colon cancer using IPA program.

Results: Totally 132,999 CCGG/8,487 CCWGG sites were identified as DMSs. The DMSs were mainly located in the intron and intergenic elements. Also 1359 CCGG/1052 CCWGG DMGs were screened respectively. Our result demonstrated that lncRNAs genes occurred frequently in DMGs. These DMGs were subjected to GO and KEGG pathway enrichment analysis. Meanwhile, the RNA expression data of 1020 DMGs were downloaded and analyzed from TCGA database. After intersection analysis, we found 14 genes were hypermethylated and downregulated and other 99 genes were hypomethylated and upregulated. The above 113 genes was subjected to determine the significant canonical pathways and gene networks involved in colon cancer using IPA program. Our results revealed significant overlap of 5 canonical pathways which connected with carbohydrate metabolism, small molecule biochemistry, cell to cell signaling and interaction, cell cycle, cell death and survival and predicted top upstream regulators including KRT14, EFNA4, HSPA6, GRK5 and ADD3. We also identified 5 significant networks associated with these genes.

Conclusion: Our study was the first to perform a MethylRAD genome-wide DNA methylation analysis for CC to acquire both CCGG and CCWGG DMSs and DMGs. This study provides potential biomarkers and therapy targets of CC. The exploration of relationship between DNA methylation and gene expression my help to better understand the molecular mechanism in tumorigenesis and development of CC.

A-312

A Polynomial Regression Analysis Model for Verification of Calibration and Linearity Performed with Several Instruments for Several Years: A Practical Example with Serum Calcium Assayed on cobas ® c501 and c311

V. M. Genta[1], E. Drumm[1], R. Murray[1], J. T. S. Francia[1], A. Schoener[2], D. Cline[3], B. Boston[1], M. Kiger[1]. *[1]Sentara Virginia Beach General Hospital, Virginia Beach, VA, [2]Sentara Independence, Virginia Beach, VA, [3]Sentara Healthcare, Norfolk, VA*

Background: The equality of analytical performance between instruments ensures their interchangeability in patient care. Our Hospital (SVBGH) receives patients from a free-standing Emergency Department (SI). Patient testing is performed with two cobas c501 instruments at SVBGH and two c311 instruments at SI. Calibration and linearity of the instruments are verified with CAP surveys and with commercially available calibration/linearity material. The homogeneity of performance for blood calcium was verified with polynomial regression analysis techniques for three years. **Methods:** Instruments: two cobas c501, two cobas c311 (Roche Diagnostics). Reagent: Calcium Gen 2 (Roche Diagnostics). Calibration/Linearity Materials: CAP Linearity Surveys for 2016, 2017, 2018; Validate® GC1, Lot #11AJ10618 (Maine Standards). The data were transferred to Minitab® (Version 17, Minitab, Inc.) statistical software and analyzed with polynomial regression analysis techniques, their diagnostics, and graphic representations. **Results:** Since multiple comparisons (P<0.0001) and Levene (P<0.0001) statistical tests showed increasing variance for increasing values of the reference value, a weighted (weight = 1/Y) polynomial regression analysis model (WPRM) was employed. While the WPRM showed no statistically significant differences between instruments (P = 0.19), there were statistically significant differences between reference material (P = 0.008) and year (P = 0.04). However, these differences were due to very small adjusted mean squares: for reference material = 0.06 mg/dL and for year = 0.05 mg/dL. The standardized deleted residuals diagnostics showed a quasi-normal distribution and a few possible outliers. The plot of the standardized deleted residuals by the observation order did not show any identifiable patterns. The plot of the standardized deleted residuals by the fitted value showed a quasi-linear distribution as demonstrated by the locally-weighted scatterplot smoother for year, instrument, and reference material. The pure error test showed a statistically significant lack of fit (P < 0.0001). This was due to a very small pure error (0.002 mg/dL). The plot of the standardized deleted residuals by the leverage (HI) showed a few potential outliers (standardized deleted residuals>|3|) and no influential observations (HI<0.05). The plot of the differences between the reference values and the observed values showed that for reference values between 0.5 - 15 mg/dL, all differences were within the allowable total error (CLIA criterion: target value ± 1 mg/dL). For values between 15 - 22 mg/dL, a few differences exceeded the -1 mg/dL target. **Conclusions:** The weighted polynomial regression model showed that the regression lines for instrument, year, and reference material were linear and similar for three years. The statistically significant differences for year and reference material regression lines were due to very small mean sum of squares error in relation to the total allowable error. Consequently they were not significant for either QC or clinical practices. The differences between observed values and reference values were within the CLIA's criterion. Consequently the cobas instruments could be used interchangeably for calcium patient assays. Since the statistical techniques employed in these studies were computationally intense, the availability of appropriate statistical software, such as Minitab, was of paramount importance for data analyses and their graphic representations.

A-313

Laboratory Investigation of Oncogenic Human Papillomavirus Genotypes: Focus on Multiple Infections and Age

J. Kim[1], J. Jeon[2], J. Kim[2]. *[1]Department of Laboratory Medicine, Dankook University College of Medicine, Cheonan, Korea, CheonAn, Korea, Republic of, [2]Department of Biomedical Laboratory Science, Dankook University College of Health Sciences, CheonAn, Korea, Republic of*

Background: Cervical cancer is the leading cause of cancer-related deaths in the female population. HPV genotypes 16 and 18 account for 70% of cases of cervical cancer. It is known that HPV infection is associated with the development of cervical carcinoma and of its precancerous lesion, early intervention can effectively prevent cervical cancer. Therefore, it is essential to evaluate the prevalence of HPV types.

Methods: The HPV detection and genotyping were performed using the AnyplexTM II HPV28 Detection Kit (Seegene, Seoul, Korea) and the CFX96 real-time thermocycler (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions.

Results: A total of 7,874 consecutive cervical swab specimens were collected and positive detection rate was 18.5%. The average age of total patients was 38.6 ± 8.55 years and most commonly detected virus types were 52 (n = 223), 68 (n = 185), and 39 (n = 154). In positive specimens, 70.4% were single infections, 30.6% were multiple infections. The ratios of multiple infections to single infections for various age groups were 49.7% for patients in their 20s.

Conclusion: The incidence of HPV and the average age of infected persons were similar to the global averages. Whereas types 16 and 18 are frequently detected in the international community, type 52 was particularly common in this study. In addition, the need for intensive management in young women was clear based on the high incidence of HPV and the high frequency of multiple infections.

A-314

Use of Machine Learning to Determine Relevant Metabolic Syndrome Chemistry and Hematology Tests Associated with Increased Waist Circumference in NHANES Data(1999-2014)

Y. Qiu[1], F. Bellavance[2], G. Cembrowski[3]. *[1]University of Alberta, Edmonton, AB, Canada, [2]HEC Montreal, Montreal, QC, Canada, [3]University of Alberta, CC Quality Control Consulting, Edmonton, AB, Canada*

Introduction: Laboratory testing is becoming more complex as as we uncover the relationships between pathophysiology and the numerous established and newer analytes. In metabolic syndrome, usually in obese, hypertensive individuals with increased waist circumference (WC), elevated triglycerides and glucose and reduced high density lipoproteins coexist in a pro-inflammatory and prothrombotic state. Even hematology is affected. WC is the best indicator of upper abdominal obesity with identical WC limits proposed for men and women. As we develop harmonized reference intervals, it is important to mitigate the bias of incorporating larger WC individuals. We have used machine learning to classify the tests that are the most affected by WC.

Methods and Materials: We used the regression trees machine learning technique to study the chemistry and hematology analytes of subjects enrolled in the US National Health and Nutrition Exam Survey (NHANES) from 1999 to 2014. We analyzed the data set by Ethnicity and Gender as well as by Gender.

Results: The NHANES data set contains 6,812 male and 7,357 female Mexican Americans, 5,615 male and 5,828 female nonHispanic Blacks and 10,090 male and 10,368 female nonHispanic Whites.The Table shows the WC-associated analytes for the Gender analysis. Hematology analytes, including iron are shaded. In both men and women, the most important chemistry decision tests are uric acid, albumin, glucose and triglycerides. It is interesting that ALT is important in both men and women, but AST is unimportant in men. Mean corpuscular volume (MCV) and white count (WBC) are the most important hematology discriminators.

Discussion: These findings are extremely important when unbiased harmonized reference intervals are being developed. If the relevant WC tests are considered together, physicians as well as their patients will receive more interpretable and actionable laboratory interpretations. Ultimately, machine-learning will guide the creation of more effective result interpretations.

Tree: males age 24+ (RxR=0.266)			Tree: females age 21+ (RxR=0.352)		
Test	Splitting Rules	Importance	Test	Splitting Rules	Importance
Uric Acid	10	1.00000	Uric Acid	7	1.0000
Glucose	11	0.85042	Albumin	10	0.9105
Albumin	12	0.84963	Triglycerides	9	0.5086
Triglycerides	11	0.73710	ALP	6	0.4810
ALT	11	0.48031	Glucose	15	0.4306
Ethnicity	6	0.42483	MCV	4	0.3457
GGT	2	0.36420	WBC	3	0.3312
WBC	4	0.27213	GGT	7	0.3222
MCV	2	0.25591	Iron	9	0.2749
Neutrophil#	2	0.25275	Ethnicity	6	0.2451
LD	4	0.22704	Monocytes%	3	0.2377
RDW	4	0.20871	RDW	5	0.1991
Creatinine	2	0.19639	Neutrophil#	1	0.1987
RBC	2	0.17427	LD	4	0.1853
MCH	1	0.15199	ALT	4	0.1747
Total Protein	1	0.14568	MCH	4	0.1667
HGB	1	0.12406	AST	2	0.1177
Lymphocyte#	1	0.11355	RBC	2	0.1138
Iron	1	0.09277	Platelets	1	0.1084
Cholesterol	1	0.08913	C3	1	0.0958
Hematocrit	1	0.08375	Phosphorous	1	0.0847
			Lymphocyte#	1	0.0835
			MCHC	1	0.0801
			MPV	1	0.0573

A-315

An Audit of Telephone Calls Received in A Singapore Public Hospital Clinical Laboratory

L. Lam, A. A. D. N. Samandika Saparamadu, P. Zeng, J. Lee. *Ng Teng Fong General Hospital, Singapore, Singapore*

Background: Laboratory-related information is typically obtained via online information resources or by calling the general laboratory enquiries telephone line at Ng Teng Fong General Hospital, Singapore. According to our staff, we often receive repetitive queries resulting in non-productive work and poor job satisfaction. Our objective is to determine the total burden of telephone calls, reasons for calling the laboratory and to identify opportunities for intervention and improvement.

Methods: A retrospective, cross-sectional study was done using data from the telephone recorder over 14 days in January 2018. We identified the type and number of calls, duration of calls and call locations. We listened to all phone call recordings, identified the queries and categorized them under six main domains: information needs, tracing results and specimens, test cancellations and rejections, Electronic Medical Records (EMR)-related, calls from internal/external stakeholders and others (such as wrong numbers and engaged calls).

Results: A total of 867 calls were recorded over 14 days and its total duration was 19.6 hours. On average, 61.9 calls were received and/or made each day and 1.4 minutes spent per call. Total number of inbound calls was 497 and its duration was 13.6 hours. Our inbound calls came from inpatient wards (62.2%), outpatient clinics (16.0%), external (15.3%) and unknown (6.5%). 43.9% of all inbound calls were queries related to information needs. Overall, an average of 1.4 hours a day and more than 1 man-day a week was spent on answering phone calls.

We identified 1454 queries and 61.5% of the queries was categorized under the domain of information needs. The rest of the queries were distributed in the following domains: from internal/external stakeholders (11.8%), EMR-related (9.1%), test cancellations and rejections (8.1%), tracing results and specimens (7.0%), and others (2.5%).

The domain of information needs was further sub-categorized into the following: sent-out tests-related (40.9%), specimen type (17.3%), special instructions/handling procedures (14.4%), test/ordering details (8.5%), send-out location (7.5%), price (4.1%), turn-around time (2.9%), contact details (2.4%) and minimal sample size (1.9%).

Conclusion: We have identified the common queries from laboratory users and the areas of opportunity to reduce the number of inbound calls by up to 40%. There is a need to educate our laboratory users on how to access laboratory-related information by other means, such as online platforms or mobile laboratory apps without calling the clinical laboratory.

A-316

Development of the Re-Marker Platform to Support Diagnostic Studies of Longitudinal (Tumor) Biomarkers

R. Moritz[1], M. Muller[1], T. M. Korse[1], D. van den Broek[1], P. Baas[1], V. van den Noort[1], J. J. ten Hoeve[1], M. M. van den Heuvel[2], H. H. van Rossum[1]. *[1]Netherlands Cancer Institute, Amsterdam, Netherlands, [2]Radboud University Medical Center, Nijmegen, Netherlands*

Background: Most tumor biomarkers routinely used in cancer care are recommended to be used in a longitudinal follow-up setting. Applications include: detection of recurrent disease after curative treatment and determining (non-)response to cancer treatment. Clinical interpretation of these consecutive biomarker results is often challenging; not supported by clinical evidence and generally based on expert opinion. To improve and support diagnostic studies of longitudinal biomarkers and to make diagnostic studies of longitudinal (tumor) biomarkers more accessible, a platform entitled “Re-marker” was developed. **Methods:** An ICT environment was designed that required: i) uploading of clinical study data including longitudinal biomarker data and clinical reference standards for response/non-response, ii) worksheet to be able to generate BReC plots (Biomarker response characteristic plots), iii) worksheet to be able to design and validate biomarker-response based medical test. **Results:** ICT infrastructure was designed based on HTML and Tableau© for the graphical interface and MSSQL and R-scripts to support dataset management and statistical/mathematical analysis. The platform allowed uploading data of various patient cohorts to support diagnostic validation studies. Data should include longitudinal (tumor) biomarkers analyzed within the patient cohorts and clinical reference standards determined at various time intervals, all related to the start of an intervention. Furthermore BReC-plots could be obtained and biomarker-response based medical tests could be designed and validated. **Conclusion:** The Re-marker platform was developed in order to support

studies investigating the diagnostic performance of longitudinal (tumor) biomarkers and the design and validation of biomarker-response based test.

A-317

Tidy Data, in a Line, PBCs and, MRs Used Amply, Saves Us All Time

F. G. Strathmann, L. Blender, R. A. Middleberg. *NMS Labs, Horsham, PA*

Background:

A 5 yr/old quality dashboard presented with extreme fatigue and headaches due to inefficient manual processing steps, limited flexibility, and an inability to consistently reproduce the underlying data. Upon initial examination, the Excel-based dashboard was found to be well designed but overly reliant on time-intensive data processing steps that were unable to scale as data accumulated. Further examination revealed excessive time required to update, lack of reproducibility and transparency, and the inability to easily perform historical data analyses beyond the 12-week span it captured. At the time of presentation, there was limited support to restructure dependent data sources resulting in a profound need to tidy and transform existing data exports. Supplemental data sources were also identified from in use 3rd party software for inclusion and the desire to have a web-accessible, user-friendly interface with minimal steps for updating were noted. Integration of geographically remote laboratories using a separate LIMS was requested.

Methods:

This vignette illustrates the all too common negative impact on productivity that results from a lack of coordinated efforts to continuously curate organizationally critical metrics. Early herculean efforts to gather and present data that were once industry leading quickly become antiquated without continuous improvement in the underlying architecture and reconsideration of past approaches. Data analytics has become an increasingly rich aspect of laboratory operations with a central goal of positioning laboratorians and organizations to make better decisions. Unfortunately, organizations often become overwhelmed as staff become consumed in the manual effort required to generate these metrics instead of identifying value added activities based on the interpretation of these data. Additionally, distinguishing the right opportunities in the ocean of data analyzed is an overwhelming task for management that can lead to misallocation of already limited resources. Here we describe our efforts to channel internal talent and desire to transform our existing, manual quality metrics into an automated, easy to use, immersive experience for laboratory quality improvement.

Results:

Included software tools included DataCamp for teaching best practices for R language analyses, the R language itself, libraries included in the "Tidyverse" package collection, Shiny and ShinyServer as a web-based platform for deployment, and a SQLite backend for data storage and easy retrieval. To allow faster and deeper insights, we implemented the use of Moving Range and Process Behavior Charts to identify true signals into the sources of variation and process characteristics, respectively. Using four simple statistical rules, we integrated the ability to monitor prospectively all processes in a flexible manner to ensure the focus remains on the highest value added work. Use of statistical rules reduced signal fatigue and allowed the identification and investigation into positive and negative variations in the data.

Conclusion:

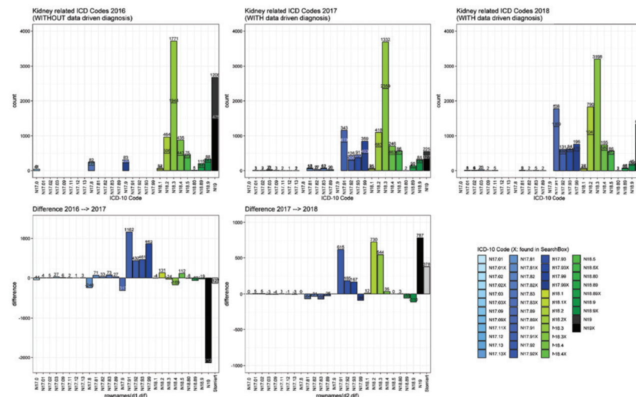
This project demonstrates a path that any organization can pursue to combine internal talent and skills with the growing community of altruistic data science experts and related tools to enhance dramatically the data analytics capabilities of laboratory staff and management.

A-318

Data Driven Precision in Healthcare: Diagnostic Accuracy and Operating Efficiencies Using Routinely Collected Health Data - Acute Kidney Injury, Chronic Kidney Disease

O. Endrich, K. Triep, N. Torbica, A. Leichtle, M. Meister, M. Fiedler. *Inselspital Universityhospital Bern, Berne, Switzerland*

Background: "The Kidney Disease: Improving Global Outcomes (KDIGO)" criteria for diagnosis and classification of kidney disease are based on a patient's current and historical values and on baseline data. For the diagnosis of acute (AKI), chronic (CKI) and acute on chronic kidney injury historic measurements of creatinine and back calculation might be required and affords an interpretation of several laboratory values over a certain period. Therefore, the process of diagnosis is complex when conducted manually. Problem: Misclassification of the correct diagnosis and the insufficient staging of the severity of a case impairs the choice of therapeutic approach and the patient's outcome. **Method:** The method of this study provides a data-driven method to assign diagnoses by extracting laboratory and administrative real-time and retrospective data from the hospital's Clinical Data Warehouse. Delta creatinine, baseline values and admission and discharge data are analyzed. A KDIGO based SQL algorithm applies specific ICD codes for AKI and CKD to inpatient stays. To measure the effect on diagnosis, Text Mining on discharge documentation is conducted. **Results:** The results displayed show the impact on first the rising number of diagnosis and second the rising precision in documentation and ICD coding. **Conclusions:** The higher validity and precision of diagnosis will not only improve the quality of documentation and data but will also improve specific and timely treatment. Measuring patients' outcome (morbidity, period of return to baseline, length of inpatient stay) will be the next step of the project.



A-319

A Statistical Analysis Solution to Measure the Impact of Sample Processing Methods on Diagnostic Laboratory Turnaround Time*

L. Zheng[1], G. Cockerham[1], M. Heydlauf[1], A. Bhalla[2], J. Hoffman[2], D. Peterson[2]. [1]*Siemens Healthineers, Cary, NC,* [2]*Siemens Healthineers, Flanders, NJ*

Background: One of the objectives of diagnostic laboratories is to satisfy turnaround time (TAT) commitments. It is imperative that laboratories process samples efficiently. In a laboratory with automation tracks, there are two processing methods for sample centrifugation: spun on-track vs. spun off-track. Similarly, there are two methods for loading samples: track-loaded vs. front-loaded. In practice, the selection of processing methods is highly subjective. Laboratories need a way to determine the TAT impact between different processing methods.

Methods: Our solution for comparing TAT of sample processing methods consists of data visualization and statistical hypothesis tests. The first component of the solution is an interactive visualization of the TAT distribution as a boxplot. The second component consists of hypothesis tests to determine if the difference in TAT between sample processing methods is statistically significant. The null hypothesis of the first t-test is that the average TAT of samples spun on-track and samples not spun on-track is equal. Similarly, the null hypothesis of the second t-test is that the average TAT of samples track-loaded and samples front-loaded is equal. This solution outputs results of both t-tests. To demonstrate the solution's capabilities, we created a simulated data set of 76,506 test orders. For each test order, we randomly assigned a sample centrifugation method and a sample loading method. We assigned four different TAT distributions to each combination of centrifugation method and loading method, respectively. Based on the processing methods of each test order, we then randomly generated a TAT from that assigned TAT distribution.

Results: In the simulated data set, visual inspection indicated the standard deviation of TAT of track-spun and track-loaded options is smaller than that of not-track-spun and front-loaded options, respectively. As part of this study, we rejected the null hypothesis and concluded that, in this simulated data set, the average TATs for the two centrifugation methods are statistically different; similarly, the average TATs for the two loading methods are statistically different.

Conclusions: Our solution uses both data visualization and statistical hypothesis tests to help laboratories make informed decisions about sample processing. Laboratories can potentially use the data visualization to comprehend the TAT distribution and the hypothesis tests to determine if different processing methods imply statistically significant TAT differences. Laboratories can then potentially use this quantified insight to standardize operations using the most efficient processing methods.

Disclaimer:

This study was completed as part of product development for Atellica® Process Manager. The first component of this solution is not yet released and is planned to be released as part of Atellica Process Manager Version 2.1. Research for the second component occurred during the feasibility phase of product development for Atellica Process Manager Version 2.1 and is also not yet released.

* This solution and the Atellica® Process Manager are in development. Not available for sale. Features listed are development design goals. Future availability cannot be guaranteed.

HOOD05162002985712

A-320

The Average of Delta: Monitoring Assay Performance through the Use of the Mean Intra-Individual Delta

M. A. Cervinski[1], E. Xu[2], G. Cembrowski[3]. *[1]Dartmouth-Hitchcock Medical Center, Lebanon, NH, [2]University of Manitoba, Winnipeg, MB, Canada, [3]University of Alberta, CC Quality Control Consulting, Edmonton, AB, Canada*

Background: Due to the limitations of traditional quality control (QC) materials and techniques, patient based real-time QC (PBRTQC) techniques are needed to continuously monitor assay performance in clinical laboratories. Patient based QC strategies such as the delta check are widely used and other techniques such as the moving average (MA) are becoming more commonplace. The MA, while useful for detection of systematic error (SE), is limited in its ability to detect the onset of SE for analytes with a skewed distribution and is less able to detect SE in hospitalized inpatient populations. A combined delta check and moving average, or Average of Delta (AoD) could be a useful QC metric to monitor assay performance for skewed analytes and for hospital based laboratories. Here we describe a new PBRTQC metric, the AoD that monitors the mean difference between pairs of consecutively drawn patient results. The objective of our study was to use existing patient data to assess the ability of the AOD to detect the onset of artificially induced SE

Methods: Using a database of 4.2 million chemistry test results spanning 638 days, individual arrays of paired patient results collected within 20 - 28 hours of each were generated for each analyte in our study. Parameters for the AoD protocols were generated using a simulated annealing algorithm in MatLab (Mathworks, Natick, MA) to select the number of pairs of patient results to average (Np) and truncation limits to eliminate large deltas that could disproportionally affect the average delta value. Control limits for each analyte were determined by taking 2.5 times the standard deviation of the delta values at 24 hours. Following the generation of AoD parameters we simulated error by adding SE at fixed intervals to the arrays of paired patient results for albumin, calcium, chloride, creatinine, magnesium, sodium, phosphorus, potassium, and total protein. Protocol performance was determined by calculating the average number of delta to detection (ANDD) for each analyte.

Results: The AoD readily detected induced SE equal to the AoD control limits. For analytes such as sodium and chloride the ANDD were 18 or 10 pairs of patient results, respectively. For total protein a negative SE equal to 0.75 g/dL was detected with an ANDD of 6.9 pairs of results. The AoD was also able to rapidly detect an SE of 0.5 g/dL in albumin with an ANDD of 7.6 pairs of patient results. The Np needed per analyte was varied but low, with as few as 5 pairs of results needed for the total protein AoD calculations.

Conclusion: We have demonstrated that the AoD is capable of rapidly detecting SE. AoD is useful for error detection in assays commonly used to monitor hospitalized patients and is complimentary to other PBRTQC techniques such as MA. The limitation of AoD is the need for pairs of patient results, however for hospitals serving a large inpatient population, repeat daily testing is commonplace. The incorporation of AoD into middleware or instrument software packages would improve detection of the onset SE.

A-321

Integrating Laboratory and Clinical Data-A Framework for Improving Reimbursement of Genomic Testing

L. Anderson, S. Denham, P. Goede. *Xifin, San Diego, CA*

Background: The rapid expansion of genomic testing, particularly NGS panels that identify numerous genomic variants of proven and emerging clinical utility, has led to some uncertainty about their appropriate use, interpretation, and clinical utility. Uncertainty surrounding clinical utility leads to claims denials which are appealed through a lengthy and often costly process that may or may not result in successful reimbursement. Thus, the financial responsibility of non-reimbursed genomic testing is either absorbed by the testing laboratory or transferred to the patient. Health informatics systems that curate laboratory, clinical and financial data can help to streamline the appeals process by providing access to critical clinical information needed to establish medical necessity in the short term, as well as allowing laboratories access to real world data to establish evidence-based coverage for the long run.

Methods: The billing records from 100 independent laboratories were reviewed to determine the proportion of molecular and NGS tests (based on CPT coding) that were denied on initial submission for reimbursement in 2018. The proportion, time-to-adjudication and added costs of successful appeals was determined after manual submission of clinical documentation with billing claims upon appeal.

Results: Approximately 80% of NGS and 40% of molecular services were initially denied. After appealing with clinical information included, approximately 55% of denials were successfully overturned. The average appeal process was completed in 60-120 days, depending on complexity and the costs incurred to labs and payers was tens to hundreds, and tens to thousands of dollars respectively.

Conclusion: Denials can be successfully adjudicated by submitting clinical information. Appeals and denials due to the lack of clinical utility are costly and time consuming for labs and payers. Health informatics systems that integrate diagnostic, clinical and financial data can streamline the claims adjudication process by automating the extraction key clinical data including CPT and ICD10 codes, and treatment plans in the short term and outcomes data that can used for coverage policy decision making in the long term.

Tuesday, August 6, 2019

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

Animal Clinical Chemistry

A-323

Use of Sebia Capillarys 2 Capillary Electrophoresis to Measure Hemoglobin A1c in Rhesus Macaque Monkeys (*Macaca mulatta*)

D. F. Stickle[1], A. E. Sarfaty[2]. [1]*Jefferson University Hospitals, Philadelphia, PA,* [2]*Yale School of Medicine, New Haven, CT*

Background: Non-human primate species are expected to have close structural analogues of human hemoglobin A. Aside from boronate affinity methods for separation of glycated from non-glycated hemoglobin, suitability of a given method to measure %A1c in non-human species must be determined empirically. We investigated whether the Sebia capillary electrophoresis system (CE, Capillarys 2 system) could provide %A1c measurements for Rhesus macaque monkeys (*Macaca mulatta*). **Methods:** Venous whole blood K-EDTA samples were collected and stored frozen until analysis. Samples were run for A1c analysis using the Sebia Capillarys 2 capillary electrophoresis system. Hemoglobin variant analysis was also performed, using the Trinity Biotech Ultra2:variant HPLC analyzer. **Results:** A total of nine monkey samples (M) were evaluated; three from subjects known to be diabetic according to clinical criteria and glucose measurements, and six from subjects not known to be diabetic. In normal human subjects, CE produces four electrophoretic peaks, corresponding to A1c, other A, A0, and A2. %A1c is derived from the ratio R = A1c/(A1c + A0), according to a linear calibration curve of %A1c vs. R for calibrator samples. CE profiles for M produced only three peaks, consistent in scale and in retention time with expected A1c, non-A1c A, and A0 peaks. The equivalent of an A2 peak was missing in all M samples, consistent with the fact that Old World monkeys do not produce the equivalent of a delta chain associated with hemoglobin A2. Because of the missing A2, CE identified the M profiles as abnormal, and did not calculate the %A1c. However, the calibration curve for %A1c vs. R (%A1c = m R + b) can be calculated from normal human subjects results. Given m and b, the R data for M samples can also be converted to %A1c. For non-diabetic M samples (n = 6), the %A1c results range was 4.0-4.7 % (NGSP, human reference interval: <5.7%), or 20-28 mmol/mol (IFCC, human reference interval: <39 mmol/mol);. For diabetic M samples (n = 3), the %A1c results range was 6.1-12.1 % (NGSP), or 43-115 mmol/mol (IFCC). Hemoglobin variant analysis by HPLC showed that 4 of the 9 M samples produced identical chromatograms having two major peaks rather than one, indicating that these subjects were heterozygous for a common hemoglobin A variant. The presence of this variant was not evident in any of the CE chromatograms. **Conclusions:** CE can provide separation of A1c and A0 to permit determination of %A1c in Rhesus macaque monkeys, based on manual calculation of the ratio R and the calibration curve. However, in comparison to human specimens, the relationship of %A1c to average glucose in M is likely to be affected by the shorter lifespan of erythrocytes (100 days). Moreover, the presence of a common hemoglobin A variant may also affect erythrocyte lifetimes in individual M subjects. M samples provided examples in which CE could not detect the presence of a hemoglobin variant.

A-324

Level of Serum Lipid in Ovarian Cancer Patients: A Hospital Based Study

N. K. Yadav[1], R. Pandey[2], D. R. Pokharel[1], P. Pandit[2], M. Lamsal[2], N. Gautam[2], N. Chhimal[2], S. K. Baral[3]. [1]*Manipal College of Medical Sciences (MCOMS), Pokhara, Nepal,* [2]*Pokhara University, Pokhara, Nepal,* [3]*B.P. Koirala Memorial Cancer Hospital, Bharatpur, Chitwan, Nepal*

Background: Lipids are required for various functions like energy production, signaling, cell growth and division. Alterations in lipid metabolism cause several diseases and most important are atherosclerosis, hypertension, diabetes, obesity and cancer. Ovarian cancer is a leading cause of death in women and the incidence of ovarian cancer is (1.9) per 100,000 populations in Nepal. The aim of this study is to see level of serum lipids in ovarian cancer.

Methods: A total of 17 samples were collected from cancer ovarian cancer patients and 50 samples collected from healthy individuals. The samples were analyzed using automated chemistry analyser in the biochemistry laboratory at B.P. Koirala Memorial Cancer Hospital, Chitwan, Nepal from 28th of October to 16th of November, 2017. The data were analyzed using SPSS 16.

Results: The ovarian cancers were 29.41% in age group 50-59 years followed by 23.52% in age group 60-69 years. Similarly, 17.64% in age group 40-49 years and <30 years and least in 11.76% in age group 30-39 years. The cancers were higher in age group 50-59 years. The mean ± SD of serum total cholesterol, triacyglycerol, HDL-C, VLDL-C, LDL-C were 128.59±32.76, 139.94±34.92, 35.88±12.58, 27.9±6.98 and 66.17±22.22 respectively in cancer patients. Similarly, the mean ± SD of total cholesterol, triacyglycerol, HDL-C, VLDL-C, LDL-C were 178.16±29.74, 175.60±61.59, 44.80±6.61, 33.77±11.40 and 98.57±25.43 respectively in healthy control.

Conclusion: The level of serum lipids were decrease in cancer patients compare to healthy control.

A-325

Dietary Quercetin Modulates Ouabain-Sensitive Na^+/K^+- Atpase in Mammalian Brain Model

A. T. Ogundajo. *Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria*

Background: The Na+, K+-ATPase is also known as the sodium pump, is a ubiquitous transmembrane enzyme that transports Na^+ and K^+ across the plasma membrane by hydrolyzing ATP. Quercetin is one of several naturally-occurring dietary flavonoids compounds, ubiquitously found in fruits, vegetables, herbs, tea, wine and as supplement

Methods: Male adult Wistar rats (200-250 g) were randomly divided into the OR group (oral), IP group (Intraperitoneal), SC group (subcutaneous) and IV group (intravenous). These groups were administered with quercetin at a dose of 50 mg/kg once daily for five (5) days based on route of administration. Quercetin was pre-dissolved in ethanol. Animals were anesthetized with ether and euthanized by decapitation, the whole brain was quickly removed and placed on ice and the homogenate was prepared in 0.05 M Tris-HCl, pH 7.4 and the supernatant was used for the assay of Na^+/K^+- ATPase. Controls were carried out under the same conditions with the addition of 0.1 mM ouabain. Na^+/K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphorous (Pi) was measured by the method of Fiske and Subbarow .

Results: This present study also showed that quercetin has inhibitory activity on cerebral sodium pump in a concentration-dependent manner irrespective of the route of administration while the effect was highly significant in IP group

Conclusion: Quercetin modulates Na^+/K^+- ATPase activity in mammalian brain model by exerting dose -dependent inhibitory effect irrespective of the route of administration.

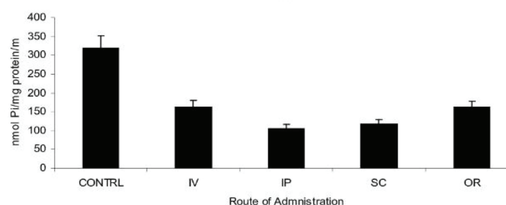


Fig 1:Effect of Quercetin on cerebral Na+/K+-ATPase in-vivo. . Data are expressed as mean±SD and represent independent experiments performed in triplicate

A-326

Effects of *Uvaria chamae* Root Extracts on Cortisol, Insulin, and Testosterone in Normal and Streptozotocin-Induced Diabetic Rats

F. E. Olumese[1], I. O. Onoagbe[1], F. O. Omoruyi[2]. [1]*University of Benin, Benin City . Nigeria, Benin, Nigeria,* [2]*Department of Life Sciences, Texas A&M University,, Corpus Christi, TX*

Background: *Uvaria chamae* is a medicinal plant that is used in many parts of the world in the treatment of diabetes and many other diseases. *Uvaria chamae* contains some chemicals like C-benzylated monoterpenes, aromatic oils, flavanones, and C-benzylated dihydrochalcones. There is paucity of information on the use of this plant

extracts in the treatment of diabetes scientifically. In this study, we evaluated the serum levels of glucose, insulin, testosterone, cortisol and histopathological changes in the pancreas of normal and diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* root.

Methods: Thirty-six Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 ± 7.20 g]: Normal Control (Healthy rats received de-ionized water); Normal plus Aqueous Extract (Normal rats received aqueous extract); Normal plus Ethanolic Extract (Normal rats received ethanolic extract); Diabetic Control (Diabetic rats received de-ionized water); Diabetic plus Aqueous Extract (Diabetic rats received aqueous extract), and Diabetic plus Ethanolic Extract (Diabetic rats received ethanolic extract). Diabetes was induced using a single injection of streptozotocin (Sigma-Aldrich, 60 mg/kg body weight in 0.05 M-citrate buffer, pH 4.5) intraperitoneally. Normal and diabetic rats were administered the aqueous or ethanolic extract (300 mg/kg body weight per day) for 35 days. Animals were then euthanized by decapitation and blood collected for glucose, and hormonal assays. The pancreas was collected and preserved in buffered formalin for histopathological evaluation.

Results: The blood glucose levels decreased by 38% and 53% in the diabetic rats administered aqueous or ethanolic extract respectively compared to the increase in the diabetic control (45%). There was a significant decrease in serum glucose/insulin ratio and upregulation of insulin in the diabetic animals administered aqueous or ethanolic extract when compared to the diabetic control. The cortisol levels were significantly decreased in the diabetic animals administered aqueous or ethanolic extract, while testosterone was significantly increased in the diabetic rats treated with ethanolic extract when compared to diabetic control. The pancreas of normal control groups treated with aqueous or ethanolic extract showed resurgent pancreatic islet cells compared to the untreated normal control. Similarly, the diabetic groups treated with aqueous or ethanolic extract showed resurgent pancreatic islet cells compared to the diabetic control group.

Conclusion: The resurgence of pancreatic islet cells and the enhanced hormones are indicative of extracts potential benefits in the management of diabetes and its associated complications.

A-327

Comparison of Commercially Available Multiplex Rat Cytokine Methods

T. Philip, K. Schorle, W. Glaab, D. Thudium. *Merck & Co., Inc., West Point, PA*

Background: The increase of therapeutic biologics and vaccines in drug development has increased the demand for cytokine measurements in non-clinical species. However, available cytokine assays for animal samples are notoriously lacking in standardization, consist of different antibody pairs, and values vary significantly across vendors. To identify the most reliable assay, we examined several rat multiplex cytokine kits with the intent of finding the best method for measuring frequently requested cytokines. In addition, rat strain, sample type, collection site, control and positive samples were investigated.

Methods: To investigate the effect of sample collection site on cytokine values, blood was collected from three sites (jugular vein, *vena cava*, tail vein) into EDTA plasma or serum tubes. Matched serum and EDTA plasma sets from control Wistar rats were collected from jugular vein and *vena cava* sites and serum and EDTA plasma were collected from the jugular of Sprague Dawley (SD) rats. Positive plasma samples were obtained from *in vivo* treatment with agents known to induce cytokines. IL-6, TNF-a and IFN-g were analyzed on the Bio-Plex 200 using magnetic microsphere-based rat cytokine methods from Bio-Rad (Bio-Plex Pro™), Millipore (Milliplex®), Thermo Fisher (ProcartaPlex™), and R&D Systems (Magnetic Luminex® Assay). Additionally, the Meso Scale Discovery (MSD) Proinflammatory Rat V-PLEX kit (multi-spot 96 well format) was evaluated.

Results: The performance of the assays varied considerably, and their performance was further influenced by the sample collection site and the sample matrix examined. Initial evaluation of the Millipore, R&D Systems, and Thermo Fisher rat assays identified issues of sensitivity, or dilutional linearity that did not warrant further investigation; thus, the evaluation was mainly focused on the Bio-Rad and MSD kits. While cytokine values for both male and female Wistar rats were comparable across these platforms, cytokine values were relatively higher and more variable using the Bio-Rad rat kit as compared to the MSD kit. Furthermore, serum samples were more variable than EDTA plasma samples, and vastly lower cytokines values were noted for terminal *vena cava* EDTA samples compared to interim jugular or tail vein collections. Finally, serum and plasma cytokine values for Wistar and SD rats were similar.

Conclusion: Our comprehensive evaluation of sample type, collection site, and available cytokine assays demonstrated that the least variable and most reliable data was generated from *vena cava* EDTA plasma samples. Rat serum resulted in considerably higher and more variable values. The Bio-Rad kit provided consistent data for the terminal EDTA plasma samples; however, interim jugular samples were highly variable. The MSD platform demonstrated the lowest and least variable values, and could be considered if interim sampling (e.g., jugular) is needed. Although our findings suggest guidelines for cytokine measurements in rat samples, careful monitoring of the performance of existing assays is recommended due to the potential variability observed with these cytokine assays.

A-328

Analysis of Serum HDL Subclass in Mouse Obesity Models by Cation-Exchange Chromatography

R. Shinohata[1], M. Shibakura[1], T. Ueno-Iio[1], Y. Arao[1], S. Watanabe[1], S. Hirohata[1], M. Okazaki[2], S. Usui[1]. [1]*Okayama University Graduate School of Health Sciences, Okayama, Japan,* [2]*Professor Emeritus at Tokyo Medical and Dental University, Tokyo, Japan*

Background: Major serum lipoproteins in rodents are not LDL but HDL unlike human. Mouse obesity models, *ob/ob* and *db/db*, are well known to have increased HDL-cholesterol (HDL-C) levels, especially along with apoE-rich HDL-C, but quantitative determination of apoE-rich HDL-C has not been available. We recently reported an analytical method for apoE-rich HDL-C determination in human sera, using a cation-exchange chromatography. In this study, we applied this technique to analyze mouse serum HDL, and compared apoE-rich HDL-C levels between wild-type, *ob/ob* and *db/db* mice.

Methods: Male 10-week old wild-type (C57BL/6J), *ob/ob* and *db/db* mice were purchased from Charles River (n=3/group). All mice were fed standard chow diet. HDL fractions were isolated from whole sera by the polyethylene glycol (PEG) precipitation method. For quantitative determination of apoE-rich HDL-C levels, the PEG supernatant was applied into a cation-exchange column (HiTrap SPHP, GE healthcare) equilibrated with 10mM MOPS buffer (pH 7.0) containing 40mM magnesium acetate. Bound HDL (apoE-rich HDL) to the column was eluted by 10mM MOPS (pH 7.0) containing 1.0M sodium acetate, and detected by an on-line enzymatic reaction for cholesterol. Protein and mRNA expression of hepatic SR-BI (scavenger receptor B1) and ABCA1 (ATP-binding cassette transporter A1) were determined in the liver tissue.

Results: Non-HDL lipoproteins from mice were selectively precipitated by the PEG method, and apoE-rich HDL remained almost completely in the PEG supernatant. The bound HDL (apoE-rich HDL) to the SPHP column eluted at the peak position between LDL and predominant HDL on a gel-permeation liquid chromatography (Superose 6, GE healthcare), indicating a selective separation of apoE-rich HDL from the PEG supernatant by the SPHP column. As for analysis of obesity model mice, total HDL-C levels were significantly higher in *ob/ob* and *db/db* than wild-type mice (138.0±16.0, 94.7±11.2 and 62.8±1.4 mg/dL, respectively). *Ob/ob* mice had increased apoE-rich HDL-C levels (30.8±10.0 mg/dL), compared to wild (7.1±0.5 mg/dL) and *db/db* (8.6±1.9 mg/dL) mice, but unexpectedly there was no significant difference in apoE-rich HDL-C levels between wild-type and *db/db* groups (P=0.958). Western blotting revealed that protein levels of hepatic SR-BI, which functions as an apoE-rich HDL receptor, was significantly lower in both *ob/ob* and *db/db* than wild-type mice, although there was no significant difference between *ob/ob* and *db/db* mice. Protein expression of hepatic ABCA1 was also lower in both *ob/ob* and *db/db* than wild-type mice.

Conclusion: Quantitative analysis of serum lipoproteins from rodents such as mice and rats is very difficult because of small sample volumes. We successfully applied the cation-exchange chromatography method to examine HDL subclass from individual mouse sera. This technique would be useful for the study of lipoprotein metabolisms in small animal models.

A-329

Role of RNase L in Kidney Recovery

N. Alghamdi, A. Zhou. *Cleveland State University, Cleveland, OH*

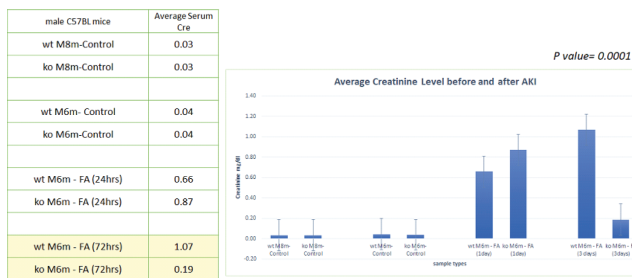
Renal diseases continues to be a prevalent problem. Current data indicate that 1% of patients admitted to hospitals are diagnosed initially with acute kidney injury(AKI), while 2-5% of hospitalized patients develop AKI secondarily. Studies in the animal model of AKI showed that epidermal growth factor(EGF) enhances recovery of renal

function and structure following acute kidney injury because it promotes renal tubular cell proliferation.

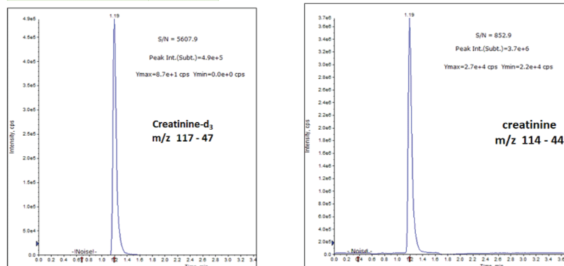
However, it has been also reported that EGF/EGFR activation contributes to the development and progression of renal diseases such as obstructive nephropathy, hypertensive nephropathy, and glomerulonephritis. High level of precursor EGF has been detected in urine although the physiological

significance reminds to be elucidated. Urinary EGF acts as a predictor of renal function outcomes since it is produced locally in the kidney. Measuring levels of EGF excreted in urine is a useful marker for many diseases and injuries. In this study, we used animal model of wild type and RNase L knock out mice to investigate the role of RNase in kidney recovery after AKI. We found that the size of kidney in RNase L deficient mice was significantly smaller than that in wild type mice at the same condition. Further investigation of the kidney function revealed that deficiency of RNase L in mice exclusively blocked the excretion of EGF. After inducing AKI and using mass spectrometry analysis to measure creatinine as a marker for kidney recovery, we found that the kidney recovery was enhanced in mice with deficient RNase L compared to wild type mice, suggesting that RNase L may involve in kidney recovery from AKI. These results greatly expand our knowledge about the role of RNase L and present a new understanding for the renal injury which will result in significant advances in elucidation of the regulatory mechanism of renal development, physiology and pathophysiology

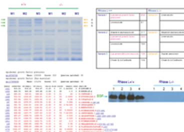
Creatinine Level



male C57BL mice	Average Serum Cre
wt M8m-Control	0.03
ko M8m-Control	0.03
wt M6m- Control	0.04
ko M6m-Control	0.04
wt M6m - FA (24hrs)	0.66
ko M6m - FA (24hrs)	0.87
wt M6m - FA (72hrs)	1.07
ko M6m - FA (72hrs)	0.19



Ion chromatograms of mouse plasma creatinine in FA treated samples measurement using LC-MS/MS. A(+/+) and B (-/-) panels show ion transition of m/z from 114 to 44 Creatinine and [^{2}H3]-creatinine were retained for 0.62 min. The intensity of each peak and the area peak are shown in table C.



Lack of RNase L exclusively blocks the excretion of EGF precursor in urine

EGF excreted into urine by a proteolytic shedding process that leading to release the entire ectodomain of a high molecular mass of 170 kDa in human and 130 kDa in mice urine[2,3]. Urinary EGF acts as a predictor of renal function outcomes since it is produced locally in the kidney. Measuring levels of EGF excreted in urine is a useful marker for many diseases and kidney injuries.

Lacking of RNase L blocks the excretion of EGF into urine. Two urinary protein bands in wild type mice were missing in the urine of mice deficient RNase L (Fig 2A). After identifying the protein bands using HPLC-MS/MS analysis, deficiency of RNase L exclusively blocked the excretion of EGF in urine (Fig B,C). The observation was confirmed by Western blot analysis using anti EGF-antibody (Abcam, Cambridge, MA) (Fig. 2D). However, we did not detect the mature EGF form in the urine by both LC/MS and immune analysis.

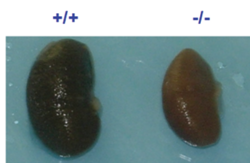


Fig. 1. Morphology of the kidneys from 18-month-old RNase L null and wild type mice with C57BJ/6 background

A-330

Validation of the qPCR from Conjunctival Swab for the Diagnosis of Canine Visceral Leishmaniasis

M. A. Almeida, T. Vital, A. P. Teixeira, D. M. Silva, B. C. de Carvalho, B. S. Dallago, G. S. C. Paz, L. Hagström-Bex, R. Pratesi, G. A. S. Romero, M. M. Hecht, N. Nitz. *Universidade de Brasília, Brasília, Brazil*

Leishmaniasis are zoonotic infections caused by the protozoa Leishmania and transmitted by Phlebotominae flies. In urban areas, dogs are the main reservoir. The canine visceral leishmaniasis (CVL) normally run asymptomatic, but this systemic disease can result in death of untreated animals. LVC diagnosis have many limitations and it is necessary to use different methods to obtain an accurate result. Molecular techniques with non-invasive samples would represent a powerful tool to improve CVL diagnosis. In this respect, the use of conjunctival swab is practical, prompt, with non-anesthetic collection and remarkable specificity and reproducibility. Herein we validated the CS-qPCR to detect Leishmania DNA in dogs from an endemic area of Federal District, Brazil. The procedure was approved by the Ethics Committee on Animal Use at University of Brasília. The canine population consisted of 124 animals residing in the studied area. The samples are tested by ELISA, DPP, culture and qPCR assays using DNA from blood (Bl-qPCR), bone marrow (BM-qPCR) and conjunctival swab CS-qPCR). The gold standard was established on positive cases for parasite culture or (DPP and BM-qPCR) or (DPP and Bl-qPCR). Statistical analysis was performed using Kappa's (κ) concordance coefficient and specificity and sensibility were calculated. The CS-qPCR results showed 80% sensitivity and 71% specificity with $\kappa = 0.72$ (CI 95%), representing an substantial agreement. CS-qPCR detected almost three times more (38.7%) positivity than recommended serological tests, (ELISA 12,1% and DPP 16,1%). Our results demonstrated that CS-qPCR may substitute the routine tests and allow a rapid and accurate diagnosis of CVL in endemic areas.

A-331

Verification of the Sysmex XN-V Multispecies Hematology Analyzer for Use in Mouse Sample Analysis

A. Love, R. Riley, J. DiPiero, D. Bounous. *Bristol-Myers Squibb, Princeton, NJ*

Introduction: The Sysmex XN-V series is an automated hematology analyzer with software that is capable of analyzing animal EDTA whole blood samples. The instrument operates under principals of electrical impedance, light scatter, and fluorescent dye binding to quantify and identify cell populations found in EDTA whole blood samples.

Objective: A verification and method comparison study was performed specifically for mouse sample analysis using the Sysmex XN-V Multispecies Hematology analyzer and the Advia 2120i Multispecies Hematology analyzer.

Method: The verification of the Sysmex XN-V Analyzer included the following components: Accuracy, Precision, Reportable Range, RBC Extended Linearity, Carryover, and Method Comparison. The Sysmex XN-V provides 3 different methods of platelet for analysis: PLT-I (Impedance), PLT-O (Optical), and PLT-F (Fluorescence). All 3 methods were compared to the Advia 2120i platelet values. Additionally, the RDW-SD parameter versus the RDW-%CV parameter was evaluated.

Results: The Sysmex XN-V demonstrated acceptable Accuracy, Precision, Reportable Range, RBC Extended Linearity and Carryover for the purpose of mouse hematology sample analysis.

Method comparison studies between the Sysmex XN-V and Advia 2120i showed variable results with the following red blood cell parameters: HGB, HCT, MCV, MCHC, and RDW-%CV. The Sysmex XN-V provides a five part white blood cell differential compared to the Advia 2120i, which provides a six part differential due to the reporting of Large Unstained Cell category.

Differences in the instrument methodologies most likely account for the findings listed above. The manufacturer offers flexibility in the Manual Gating feature to redefine cell populations in the white blood cell differential scattergram.

It was determined that PLT-F, fluorescence method, would be the reportable parameter for platelet values. This is due to due to the increased specificity of the fluorescent dye used in the method and the claim that this parameter results in less platelet clump flags, which are frequently noted in rodent samples.

It was also decided that the RDW-SD, would be the reportable parameter rather than the traditional RDW-%CV. The RDW-SD is a direct and more accurate measurement because it is independent of MCV values used in the RDW-%CV calculations.

Conclusion: It is best practice with new instrumentation, and for the reasons stated above, in-house reference ranges will be established for mouse hematology analysis using the Sysmex XN-V. The Manual Gating feature will be utilized to optimize mouse gating strategies and manual differential results will serve as the reference method.

A-332

Pharmacological Inhibition of the Metabolic Pathway that Converts Epoxy- to Dihydroxy-Metabolites of Omega-6 and Omega-3 Fatty Acids Attenuated Alcohol-Induced Liver Injury in Mice via Reduction of ER Stress

J. Warner, D. Warner, Y. Song, C. McClain, I. Kirpich. *University of Louisville, Louisville, KY*

Background: Omega-3 and omega-6 fatty acids are metabolized via the cyclooxygenase and lipoxygenase pathways, but are also substrates of cytochrome P450 (CYP) epoxygenases, which convert them to signaling lipid metabolites with potent anti-inflammatory properties. However, these epoxides are quickly metabolized by soluble epoxide hydrolase (sEH) to their corresponding dihydroxy metabolites (diols). The biological effects of diols are not well defined, and they potentially might exert both anti- and pro-inflammatory properties. sEH inhibitors such as trans-4-{4-[3-(4-trifluoromethoxyphenyl)-ureido] cyclohexyloxy} benzoic acid (t-TUCB), are being explored as a therapy for multiple pathologies, including liver diseases of different etiologies. Alcoholic liver disease (ALD) is a significant human health problem with global ramifications due to its significant morbidity, mortality, and burden on the health care system. There are no effective FDA-approved medications to prevent or treat any stages of ALD and the mechanisms involved in ALD pathogenesis are not well understood. **The goal** of the present study was to determine whether pharmacological inhibition of sEH by t-TUCB would attenuate ethanol (EtOH)-induced liver injury in mice and to identify underlying mechanism(s). **Methods:** C57BL/6 male mice were fed for 10 days with an all-liquid diet containing 5% EtOH or pair-fed with isocaloric maltodextrin (to control for energy consumption), followed by a single oral EtOH gavage (5 g/kg). Mice were euthanized 9h later and blood and liver tissue were collected. t-TUCB (5 µg/ml) was administered to mice daily with food. Plasma alanine aminotransferase (ALT) levels were measured as a marker of liver injury. Formalin-fixed, paraffin-embedded liver sections were prepared and stained with hematoxylin and eosin, terminal deoxynucelotidyl transferase dUTP nick end labeling (TUNEL), and chloroacetate esterase (CAE) for histological evaluation of liver damage, hepatic apoptosis and neutrophil accumulation, respectively. Liver RNA was isolated for determination of gene expression changes involved in pro-inflammatory and ER stress pathways. **Results:** Compared to control animals, EtOH administration resulted in liver injury, which was significantly attenuated by inhibition of sEH as evidenced by reduced plasma ALT levels in the EtOH+t-TUCB group when compared to the EtOH-administered group (102 ± 27 vs 355 ± 114 U/L, respectively, $p < 0.05$). Hepatic steatosis and inflammation (CAE-positive cells), and apoptosis (TUNEL-positive cells) were similar in EtOH and EtOH+t-TUCB treated animals. Importantly, t-TUCB treatment prevented EtOH-induced up-regulation of *Chop*, a critical gene related to ER stress, a process which has been shown to contribute to ALD when perturbed. **Conclusions:** Our data demonstrate that disruption of the metabolic pathway that converts epoxy- to dihydroxy-metabolites of omega-6 and omega-3 fatty acids attenuated EtOH-induced liver injury (as shown by decreased plasma ALT activity), and suggest that reduced ER stress might be of the mechanisms contributing to the beneficial effects of sEH pharmacological inhibition in ALD. Given that sEH inhibition most likely resulted in alterations of the bioactive epoxides and diols, further studies are required to elucidate the exact mechanisms by which they exert their effects during ALD pathogenesis.

Tuesday, August 6, 2019

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

Tumor Markers and Cancer Diagnostics

A-333

Expression Signatures of Exosomal Long Non-Coding RNAs in Urine Serve as Novelnon-Invasive Biomarkers for Diagnosis and Recurrence Prediction of Bladder Cancer

L. Du, Y. Zhan, C. Wang. *Department of Clinical Laboratory, The Second Hospital of Shandong University, Jinan, China*

Background: Expression signatures of exosomal long non-coding RNAs (lncRNAs) have been proposed as potential non-invasive biomarkers for the diagnosis and prognosis of malignant tumors. However, only few urinary exosome (UE)-derived lncRNAs are characterized as potential biomarkers in bladder cancer (BC) patients. We aimed to develop a UE-derived lncRNA panel for diagnosis and recurrence prediction of BC.

Methods: Exosomes were extracted from urine of BC patients and healthy controls and confirmed using transmission electron microscopy (TEM), Western blotting analysis, nanoparticle tracking analysis (NTA) and flow cytometry. Then, quantitative real-time polymerase chain reaction (qRT-PCR) was performed to screen and evaluate the expressions of eight candidate lncRNAs in a training set (208 urine samples) and a validation set (160 urine samples). Multivariate logistic regression model was used to establish the selected lncRNA panel. Receiver-operating characteristic (ROC) curves were employed to evaluate the performance of the panel. In addition, the correlation between lncRNAs and recurrence-free survival (RFS) of non-muscle-invasive BC (NMIBC) patients was evaluated to explore prognostic value.

Results: Three UE-derived lncRNAs (MALAT1, PCAT-1 and SPRY4-IT1) were identified to be significantly up-regulated in BC, and a three-lncRNA panel based on this result was established for BC diagnosis. The performance of such three-lncRNA panel was verified with an area under the ROC curve (AUC) of 0.854 and 0.813 in the training set and validation set, respectively, which was significantly higher than that of urine cytology (0.619). In addition, Kaplan-Meier analysis suggested that the up-regulation of PCAT-1 and MALAT1 was associated with poor RFS of NMIBC ($p<0.001$ and $p=0.002$, respectively), and multivariate Cox proportional hazards regression analysis revealed that PCAT-1 ($p=0.018$) and the tumor stage ($p = 0.036$) were independent prognostic factors for the RFS of NMIBC ($p = 0.018$).

Conclusions: Our findings indicated that UE-derived lncRNAs possessed considerable clinical value in the diagnosis and prognosis of BC.

A-334

Five-gene Set in Blood Cells Act as Novel Potential Biomarkers for Non-Small Cell Lung Cancer

L. Xie. *Shandong Cancer Hospital and Institute, Jinan, China*

Background: We aimed to screen differential genes in the blood cells and validate their diagnostic values in NSCLC.

Methods: The blood cells from 166 NSCLC and 148 healthy donors were collected underwent total RNA isolation. RNA-seq technology was used to analyze the transcriptome of peripheral blood cells. The mRNA expression levels of differential genes were analyzed by RT-qPCR. Statistical analyses were performed using Prism and SPSS by Mann-Whitney nonparametric test, Kruskal-Wallis test and one-way ANOVA..

Results: RNA-seq technology screened 95 differentially expressed genes (DEGs), wherein 15 genes were up-regulated, and 80 genes were down-regulated. Of these, 5 genes were selected for the further analysis, wherein two were up-regulated (*GPX1, PRELID1*) and three were down-regulated (*MAP3K7CL, BCL9L, PCSK7*). RT-qPCR was performed in 303 samples (159 NSCLC, 144 controls).The five-gene panel showed significant differences ($P<0.001$) in the expression levels between NSCLC and healthy samples. ROC curves of the panel revealed an AUC of 0.843, with a sensitivity of 74.2% and specificity of 79.9%. *GPX1, PRELID1,* and *PCSK7* genes distinguished early-stage NSCLC patients from healthy group ($P<0.05$). *BCL9L* and *PCSK7* were associated with lymph node metastasis ($P=0.0008$, $P=0.0212$) and *PRELID1* was associated with distant metastasis ($P=0.0408$). In addition, the down-regulated gene *BCL9L* was associated with chemotherapeutic effect.

Conclusion: The present study provided a systematic description of gene expression profiling in peripheral blood. Remarkably, these five genes might be a possible candidate gene for the diagnostic biomarker of NSCLC and provides the basis for further biological and functional investigations.

A-335

Tumor-Associated Exosomal miRNA Biomarkers to Differentiate Metastatic Versus Non-metastatic Non-Small-Cell Lung Cancer

L. Xie. *Shandong Cancer Hospital and Institute, Jinan, China*

Background: Exosomal microRNAs (miRNAs) are proposed to be excellent candidate biomarkers for clinical applications. However, little is known about their potential value as diagnostic biomarkers for metastatic non-small-cell lung cancer (NSCLC).

Methods: In this study, microarrays were used to determine distinct miRNA profiles of plasma exosomes in a discovery cohort of healthy donors, metastatic NSCLC and non-metastatic NSCLC patients. Three potential candidate miRNAs were selected based on the differentialexpression profiles. The discovery set data was validated by quantitative real-time PCR (qRT-PCR) using a validation cohort.

Results: NSCLC patients (n=80) and healthy controls (n=40) had different exosome-related miRNA profiles in plasma. Results demonstrated that the level of let-7f-5p was decreased inplasma exosomes of NSCLC patients (P<0.0001). Further analysis of three differentially expressed miRNAs revealed that miR-320a, miR-622 and let-7f-5p levels could significantly segregate patients with metastatic NSCLC from patients with non-metastatic NSCLC(P<0.0001, P<0.0001, P=0.023, respectively). In addition, the combination of let-7f-5p, CEA and Cyfra21-1 generated an AUC of 0.981 for the diagnosis of NSCLC patients, and the combination of miR-320a, miR-622, CEA and Cyfra21-1 had an area under curve (AUC) of0.900 for the diagnosis of patients with metastatic NSCLC.

Conclusion: This novel study demonstrated that plasma exosomal miRNAs are promising non-invasive diagnostic biomarkers for metastatic NSCLC.

A-338

Smoked Foods Amplify the Levels of Benzo a Pyrene in Apparently Healthy Nigeria Men

F. F. ANJORIN, K. S. ADEDAPO. *UNIVERSITY OF IBADAN, NIGERIA., IBADAN NIGERIA., Nigeria*

Introduction: Prostate cancer aetiology has been linked to dietary factors. The presence of Benzo{a} pyrene which is a prototype of Poly Aromatic Hydrocarbon (PAH) in foods is linked with various methods of food preparation. The relationship between dietary factors and the level of the toxicant- PAH in men with prostate cancer in Ibadan, Oyo state, Nigeria therefore needs to be established.

Methodology: The study included thirty (30) prostate cancer patients aged 55-85 and thirty apparently healthy age matched controls. Prostate Specific Antigen (PSA), Benzo {a} pyrene (BaP) and Total antioxidant Status (TAS) were analyzed using standard methods. BaP was analyzed using high performance liquid chromatographic technique (HPLC). Data obtained were analyzed statistically as appropriate and $p < 0.05$ was considered significant.

Results: The mean value of PSA was predictably significantly higher in Prostate cancer patients compared to the controls while, surprisingly there was a significant decrease in the level of BaP in men with prostate cancer compared with the controls. Correlation analysis however showed a significant positive correlation between smoked fish intake and BaP in the both the cases and the controls. TAS was significantly higher in the controls than in prostate cancer patients.

Increased consumption of smoked foods, reduced TAS and resultant oxidative stress may contribute to the aetio-pathogenesis of prostate cancer. Antioxidant supplementation may enhance the total antioxidant status and may be of help in the management of prostate cancer.

A-339

Evaluation of Plasma Pro and Anti Inflammatory Markers in Papillary Thyroid Carcinoma before and after Thyroidectomy

J. G. de Araújo[1], B. Bertol[2], I. A. Sadissou[2], P. Sonon[2], F. Dias[2], R. H. Bortolin[1], N. L. de Figueiredo-Feitosa[2], L. C. de Freitas[2], S. R. d. H. Tarrapp[3], C. d. Ramos[3], A. D. Luchessi[1], J. C. d. Crispim[1], L. Z. Maciel[2], E. A. Donadi[2], V. N. Silbiger[1]. *[1]Federal University of Rio Grande do Norte, Natal, Brazil, [2]University of São Paulo, Ribeirão Preto, Brazil, [3]Hospital Liga Norte Riograndense Contra o Câncer, Natal, Brazil*

Background: Papillary thyroid carcinoma (PTC) is a type of well-differentiated thyroid carcinoma that is the most common thyroid malignancy. Preoperative diagnosis is inconclusive in up to 25% of thyroid nodules, and therefore the identification of potential new minimally-invasive diagnostic biomarkers of malignancy became crucial for improvement of PTC clinical management. There is a recognized association between PTC and chronic inflammation, thus the present study aimed to investigate plasma levels of pro- and anti-inflammatory cytokines and soluble HLA-G as potential biomarkers for diagnosis and/or prognosis of CPT.

Methods: The study included 85 PTC patients from whom blood was drawn before (n = 85) and after thyroidectomy (n = 77), and 80 unrelated healthy blood donors as the control group. Plasma levels of 13 cytokines (IFN-γ, TNF, IL-12p70, IL-1α, IL-4, IL-5, IL-13, IL-10, IL-17A, IL-1β, IL-6, IFN-α and TGF-β1) were measured by flow cytometry bead-based assays, and soluble HLA-G was measured by ELISA. Data were evaluated using univariate and multivariate analyses, and ROC curves.

Results: IL-4, IL-10, TNF, IFN-α and TGF-β1 levels were increased in post-thyroidectomy compared to pre-thyroidectomy (p = 0.006, p = 0.013, p = 0.045, p < 0.001, p = 0.001, respectively). Compared to controls: i) sHLA-G levels were significantly decreased after thyroidectomy (p = 0.002).; ii) IL-6 levels were increased (p < 0.001), while IL-1β, IFN-α and TGF-β1 levels were decreased in pre-thyroidectomy (p = 0.036, p < 0.001, p < 0.001, respectively); and iii) IL-5 and IL-6 levels were increased (p = 0.045, p < 0.001, respectively), while IFN-α and TGF-β1 were decreased in post-thyroidectomy (p < 0.001, p < 0.001, respectively). IFN-α and TGF-β1 levels were good discriminators between PTC patients and controls after multiple logistic regression analysis (p < 0.001, p = 0.008 respectively), and IFN-α presented the best diagnostic performance (AUC = 0.94, sensitivity = 0.92 and specificity = 0.82). Increased IL-1β and decreased IL-12p70 levels presented independent associations with larger tumors (>2.0 cm), while decreased sHLA-G levels were associated with invasion. Patients presenting poor response to treatment showed higher IL-5 and IFN-α levels when compared to patients with good response (p = 0.014; p = 0.003, respectively).

Conclusion: Our data indicate that there is a differential cytokine profile between plasma before and after thyroidectomy, and certain cytokines are associated with poor prognosis and response to treatment indicators (IL-1β, IL-5, IFN-α). However, increased sHLA-G levels were not associated with thyroid malignancy or poor prognosis. IFN-α and TGF-β1 levels seems to be good candidates to be evaluated in future studies as potential biomarkers for PTC preoperative diagnosis.

A-340

MiR-21-3p,miR-21-5p,miR-423-5p as Potential Serum Markers in Diagnosis of Breast Cancer

J. Xu[1], D. Liu[1], W. Wang[1], Y. Li[1], J. Huang[1], F. Lin[1], L. Lin[1], F. Luo[1], J. Xu[2]. *[1]the Second Afliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, 510120, China., GuangZhou, China, [2]Shunde Hospital of Guangzhou University of Chinese Medicine, FoShan, 528000, China., FoShan, China*

Objective: To study the expression of breast cancer-related miRNAs in serum of normal people and breast cancer patients, and to analyze its clinical value in the diagnosis of breast cancer. **Methods:** Serum samples from 68 patients with breast cancer and 38 normal subjects were collected. Real-time quantitative PCR (qRT-PCR) was used to detect miR-21-3p, miR-21-5p, miR-99a-5p, miR-223. -3p and miR-423-5p expression. To analyze the value of individual serum miRNAs in the diagnosis of breast cancer, and to further analyze the diagnostic value of combined detection of multiple miRNAs by statistical methods. **Results:** The expression levels of miR-21-3p, miR-21-5p and miR-423-5p in breast cancer patients were increased compared with the normal control, *P<0.01*; The expression levels of miR-99a-5p and miR-223-3p were not statistically different between the two groups. The ROC curve analysis showed that the area under the curve (AUC) of miR-21-3p in the breast cancer group was 0.739 (95% CI 0.64 - 0.83), *P < 0.0001*; the AUC of miR-21-5p was 0.838(95% 0.75 - 0.92), *P< 0.0001*; the AUC of miR-423-5p was 0.742 (95% CI 0.64 - 0.84), *P < 0.0001*. The three miRNAs of miR-21-3p, miR-21-5p and miR-423-5p were combined in the diagnosis of breast cancer with an AUC value of 0.843 (95% CI 0.76 - 0.92), *P < 0.0001*; Single and pairwise detection of miR-21-3p, miR-21-5p and miR-423-5p, *P < 0.0001.* **Conclusion:** miR-21-3p, miR-21-5p and miR-423-5p are elevated in the serum of breast cancer patients, and the expression of miR-21-3p, miR-21-5p and miR-423-5p in serum can be as an ideal marker for breast cancer diagnosis.

A-341

Determination of 4Kscore from Serum Stored on Clot

C. E. Higgins[1], Y. Dong[1], J. Weisberger[2], L. Gomella[3], J. Mark[3]. *[1]OPKO Diagnostics, LLC, Woburn, MA, [2]BioReference Laboratories, Elmwood Park, NJ, [3]Department of Urology, Thomas Jefferson University, Philadelphia, PA*

Background: The 4Kscore Test (4Kscore) quantitates the risk of aggressive prostate cancer using clinical data and laboratory measurement of total prostate-specific antigen (tPSA), free PSA (fPSA), intact PSA (iPSA), and human kallikrein-related peptidase 2 (hK2). Both tPSA and fPSA are measured using the Roche COBAS, while iPSA and hK2 are determined on the Perkin Elmer AutoDELFIA. Previous studies established the 4Kscore is stable in serum stored up to three days at room temperature (RT). Use of an un-centrifuged serum separator tube (SST) shipped from a physician's office would significantly improve patient access to the 4Kscore. This study addresses the stability of the 4Kscore in an uncentrifuged SST at RT for up to three days.

Methods: Forty-one male subjects seeking urological care were prospectively consented and enrolled based on their tPSA measurements falling into one of three categories: 1.5ng/mL-4ng/mL, 4.1ng/mL-10ng/mL, or above 10.1ng/mL. In addition, ten subjects in the 1.5ng/mL-4.0 ng/mL category were enrolled to supplement the urology clinic subjects. Five SSTs were drawn from each subject; one tube was spun within two hours according to vacutainer manufacturer instructions, the second immediately on receipt at the reference lab, and the remaining tubes on subsequent days. The serum in each tube was assayed the same day as centrifugation. All samples were assayed according to SOP for the 4Kscore at BioReference Laboratories (Elmwood Park, NJ). Results were analyzed by longitudinal regression.

Results: The changes in analyte recovery and in the 4Kscore result were clinically insignificant.

Parameter	Median value for control samples, ng/mL (95% CI)	Mean change per day stored uncentrifuged (95% CI)	P value for change
Age, years	63.2 (51.5 to 73.2)	NA	NA
tPSA	5.080 (2.198 to 20.734)	1.36% (1.09% to 1.64%)	<0.001
fPSA	0.820 (0.270 to 2.714)	-0.52% (-0.90% to 0.14%)	0.008
iPSA	0.507 (0.157 to 1.355)	1.87% (1.03% to 2.71%)	<0.001
hK2	0.076 (0.023 to 0.189)	1.83% (1.32% to 2.34%)	<0.001
4Kscore	11% (1% to 62%)	0.86 4Kscore units (0.6 to 1.1 4Kscore units)	<0.001

Conclusion:

The 4Kscore Test and the component analytes can be accurately determined from specimens stored in uncentrifuged SSTs for up to three days at RT, with no clinical difference from specimens centrifuged within two hours of collection.

A-342

Potential Role of Nuclear PD L1 Expression in Epithelial Mesenchymal Transitioned Circulating Tumor Cells as a Prognostic Marker in Prostate Cancer

J. Li, A. Satelli, I. Batth, Z. Brownlee, C. Rojas, S. Kopetz, E. A. Wagar, S. Li, Q. H. Meng. *MD Anderson Cancer Center, Houston, TX*

Background: The current FDA approved assay for detecting circulating tumor cells (CTCs) (CellSearch) relies on capture of epithelial cell adhesion molecule (EpCAM). CTC detection as liquid biopsy-based approach has shown promise in monitoring cancer patient management and prognosis of patient outcome. However, during the course of disease, CTCs undergo epithelial mesenchymal transition (EMT), while the CellSearch based method is unable to capture these CTCs undergoing EMT. Thus, enumeration of CTC cannot provide accurate information for cancer patient management. To address this gap in technology, we discovered the cell-surface vimentin (CSV) as a marker for detecting mesenchymal CTCs from prostate cancer, and evalu-

ated the prognostic value of programmed death-ligand 1 (PD-L1) expression in CTCs in prostate cancer patients.

Methods: Peripheral blood samples were collected from 12 healthy patient and 48 metastatic prostate cancer patients. Blood samples were analyzed for CellSearch-based epithelial CTC counts, and serum prostate-specific antigen (PSA) quantification. CellSearch used a cut-off threshold of 5 CTCs/7.5 mL. Further, CTCs undergoing EMT were isolated from the samples using magnetic separation with the CSV-specific 84-1 monoclonal antibody. We used the same threshold in CSV method. PD-L1 expression was detected and localized in the membrane and/or cytoplasm and nucleus in these cells. CSV positive CTCs from 30 patients were enumerated and analyzed for PD-L1 expression using flow cytometry. Cells with both nuclear and membrane expression of PD-L1 were included in the nPD-L1+ population, whereas cells with only membrane expression of PD-L1 were included in the nPD-L1- population.

Results: In comparison with CellSearch, the CSV-based method had greater sensitivity (93.3%) and specificity (94.4%) than CellSearch (53.3% and 83.3% respectively). CSV-based CTC detection alone was not associated with overall survival or progression free survival in prostate cancer patients, but nuclear PD-L1 (nPD-L1) expression was significantly associated with progression free survival (0.2739 vs. 38.39).

Conclusion: nPD-L1 could potentially be a clinically relevant prognostic biomarker for prostate cancer. Thus, use of CSV-based CTC models with the incorporation of nPD-L1 expression detection can be used for cancer risk assessment and to predict the prognosis.

A-343

Profiling Protein-Protein Interactions with Single-molecule Imaging to Determine Cancer-specific Signaling and Its Application to Precision Medicine

H. Lee[1], B. Choi[1], H. Kim[2], S. Im[3], B. Cho[4], T. Yoon[5]. *[1]Proteina co., Ltd., Seoul, Korea, Republic of, [2]Department of Physics, KAIST, Daejeon, Korea, Republic of, [3]Seoul National University Hospital, Seoul, Korea, Republic of, [4]Yonsei University Hospital, Seoul, Korea, Republic of, [5]Seoul National University, Seoul, Korea, Republic of*

Background: Protein-protein interactions (PPIs) trigger cell-signaling cascades that finally lead to proliferation, differentiation and apoptosis of cells. Dysregulated PPIs frequently result in many diseases, especially cancers. Therefore, understanding the pattern of PPIs in specific cancer may provide a novel therapeutic target to suppress cancer signaling. Here, we have shown that single-molecule pull-down and co-immunoprecipitation (co-IP) techniques can be applied to characterize PPIs in quantitative manner.

Methods: We analyzed human epidermal growth factor receptor (HER)-family signaling in various cancer models by using single-molecule techniques. As a model system to study changes in PPIs by genetic mutations, lung adenocarcinoma cell lines carrying deletion mutation on exon 19 of epidermal growth factor receptor (EGFR) gene were used. We pulled down EGFR in non-denaturing condition onto our imaging surface from 1-10 μg of cell extracts. Then, we applied single-molecule pull-down analysis to investigate the components of protein complexes by using HSP90α, GAPDH, Shc1, Cbl and Mig6 antibodies. At the same time, various EGFR downstream proteins were applied as probe proteins for single-molecule co-IP analysis. To validate the capability of single-molecule techniques as a diagnostic tool, EGFR tyrosine kinase inhibitor (TKI), gefitinib, were treated to 6 lung adenocarcinoma cell lines having EGFR-TKI sensitive mutations on *EGFR*. We also profiled EGFR PPIs and gauged EGFR-signaling metric that contains the expression level and the strength of PPIs. We further validated the predictive power of our technique in eight patient-derived tumor xenograft (PDX) models regardless of EGFR mutations. Especially, five PDXs with lung squamous cell carcinoma carried no EGFR-TKI sensitive mutations.

Results: We found that oncogenic mutation on EGFR led to the formation of large protein complexes surrounding mutant EGFR. This signalosome related to mutant EGFR reduced the dependency of oncogenic EGFR signaling on phosphotyrosine residues. Specifically, Grb2, the adaptor protein relaying EGFR signaling toward Ras/MAPK cascades, constitutively interacted with mutant EGFR in the absence of phosphotyrosines on EGFR. We also showed that the developed signaling metric was strongly correlated to EGFR-TKI dependency (Spearman's $\rho = 0.83$ in cell line models and $\rho = 0.95$ in PDXs) regardless of EGFR mutations.

Conclusions: Our approach might help to predict the efficacy of EGFR-targeted drugs from individual patients, particularly for cancer patients that lack actionable mutations.

A-344

Enabling Accurate miRNA Biomarker Discovery from Liquid Biopsies

H. Kim[1], G. Wu[1], K. Lin[1], B. Janssen[1], R. Kemp[1], Q. Okobi[2], S. Barberan-Soler[2], X. Jia[1]. *[1]Zymo Research Corp, Irvine, CA, [2]SomaGenics Inc, Santa Cruz, CA*

The gold standard of using clinical tissue biopsies for initial diagnosis is often invasive and cumbersome in nature, leading to the high impracticability of regular patient testing. Recent advances in the field of liquid biopsies through the analysis of the cell-free nucleic acids (cfNA) hold much promise to achieve the goal of early detection. The cfNA that are released from cells throughout the body circulate in blood and may serve as biomarkers that can be used to monitor health status. However, the minute amount of cfNA coupled to a lack of efficient isolation methods for cfNA serve as big hurdles for liquid biopsy research. Here we demonstrate an efficient novel workflow that results in 100-fold increased yield and up to 4-fold increased number of uniquely identified microRNA species over alternative technologies. Quantification comparison is demonstrated consistently by both RT-qPCR and fluorescence-based methods. In conjunction with a library prep kit optimized for biofluids, we show robust small RNA sequencing is possible from cfRNA isolated from just 200 μl of plasma. Read qualities are validated by high read alignments to both human genome (hg38) and annotated microRNA database (miRBase). Furthermore, the extraction chemistry provides flexibility in co-purifying or separating cfDNA and cfRNA from the same liquid sample, eliminating the need for multiple processing runs and excess sample input. In brief, coupling these isolation and library preparation technologies achieves more accurate miRNA biomarker profiling and enables liquid biopsy research to reach a level far beyond what was possible previously.

A-345

Novel P-glycoprotein Biosensor Technology Detects Six Anticancer Modulators of the Transporter through a Fluorescence-Based Drug Profiling

B. Osa-Andrews, K. Tan, S. Iram. *South Dakota state university, Brookings, SD*

Background: P-glycoprotein, the most clinically important ABC transporter protein, mediates the efflux-translocation of a several structurally unrelated solutes across plasma membranes by ATP-dependent hydrolysis. The lipid bilayer-located P-gp governs tissue defense, efficacy/toxicity of administered drugs and clinical multidrug resistance of anti-malignancies, antiretrovirals, and antiparasitic. Human Pgp-mediated multidrug resistance is responsible for failure of chemotherapy in small cell lung cancer, liver cancer and lymphomas through the active-pumping out of anticancer agents by the overexpressed transporter. Consequently, in 2017, the U.S. FDA strongly recommended that all drugs at clinical trials should be profiled for their interaction with P-gp. This recommendation and the clinical significance of P-gp provide a rationale for investigating the structure, function and ligands which interact with the transporter.

Methods: In the present study, we developed a two-color P-gp biosensor technology by fusing green fluorescence protein and red fluorescent protein with the transporter. We genetically engineered a set of six P-gp biosensors and coupled it with Fourier Resonance Energy Transfer (FRET) to determine the most FRET sensitive useful for anticancer drug-screening. To validate the localization and function of the P-gp biosensor, we transfected HEK293T cells with the recombinant plasmid for 48-hours after which the cells were incubated with 50 μM of doxorubicin. To further corroborate that the P-gp biosensor is functional, a similar experimental assay was conducted using verapamil (a known inhibitor of P-gp) in combination with doxorubicin. In both transfection methods, signals were observed using confocal microscopy. To validate efficiency of the P-gp biosensor technology, 50 anticancer drugs at concentration of 50 μM each, were screened with the P-gp biosensor through FRET experiments at 37 ^{0}C using Tris sucrose buffer.

Results: We observed that of the six cloned two-color P-gp biosensors, GR-678, localizes precisely in the plasma membrane, extracellularly exports doxorubicin and its doxorubicin mediated transport is inhibited by verapamil. Consequently GR-678 was selected as the lead biosensor for steady state FRET measurements using fluorometer model H-11, an affordable and commonly used instrument. Our results show that GR-678 displays etoposide-induced percent FRET change which increases upon inclusion of ATP, suggesting normal FRET response. In addition, we observed the highest percent FRET change in the presence of etoposide/ATP/sodium orthovanadate. This result suggests that GR-678 reports vanadate trapping of hydrolytic reactions in the

ADP-bound condition which is consistent with earlier reports. GR-678 identified six hit-drugs following the 50-drug screening of NIH anticancer library.

Conclusion: Overall, we have developed the first ever FRET-sensitive P-gp biosensor, useful for high throughput profiling of direct drug-Pgp interactions. Our transformative P-gp biosensor technology provides early-alert detection, before clinical trials, of anticancer drugs which will be susceptible to P-gp extrusion from cancer cells and whose withdrawal from trials is recommended. This innovative technology is fast, affordable, less labor-intensive; a promising alternative to the laborious MDCK-II and Caco-II monolayer vectoral assays currently used by pharmaceutical industries. This technology will transform the future of chemotherapy by enhancing combinatorial therapy and targeted therapy thereby reducing disease burden, mortality and improving the clinical course of cancers.

A-346

Apoliprotein C1(APOC1) as a Novel Diagnostic and Prognostic Biomarker For Gastric Cancer

J. Yi[1], L. Ren[2], J. Wu[1], W. Li[2], G. Du[2], J. Wang[2]. [1]*Peking Union Meical College Hospital, Beijing, China,* [2]*Institute of Materia Medica,Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China*

Background: Gastric cancer (GC) is a common malignant tumor in the worldwide, especially in China. Patients with GC have poor prognosis due to lack of delayed diagnosis and non-specific symptoms in the early stages. Up to now, there is no good biomarker to detect GC at early stage. Apolipoprotein C1 (APOC1), a component of both triglyceride-rich lipoproteins and high-density lipoproteins, is reported to be involved in numerous biological processes. In the study, we investigated if APOC1 was used as a diagnostic and prognostic biomarker for GC.

Methods: Serum from 65 GC patients and 40 healthy individuals were detected by ELISA. Expression of APOC1 protein was evaluated in both GC and adjacent issues of GC and normal tissues using tissues array by immunohistochemistry. Expression of APOC1 and clinical characteristic of GC as well as prognosis of patients with GC were analysed respectively.

Results: It was firstly found that APOC1 concentration was significantly higher in GC than that in control. Expression of APOC1 protein was also higher in GC than that in adjacent issues of GC and normal tissues using tissues array by immunohistochemistry. Besides, the overall expression of APOC1 is significantly associated with clinical stage (p=0.011), tumor classification (p=0.010), as well as with the lymph node metastasis (p=0.048). Area under the curve of receiver operating characteristic of APOC1 was 0.803. Furthermore, elevated APOC1 concentration in serum was found to be correlated with decreased overall survival ($p<0.0001$).

Conclusion: These results suggest that APOC1 in serum may be used as a potential biomarker to detect GC at early stage and predict prognosis of GC.

A-347

Platelet Activation Status in the Diagnosis and Postoperativeprognosis of Hepatocellular Carcinoma

B. Wang, W. Guo. *Zhongshan Hospital, Fudan University, Shanghai, China*

Background: The venous thromboembolism, which may be caused by increased platelet activation, is a risk factor for tumor prognosis. The aim of this study was to determine the platelet activation status for diagnosis and predicting postoperative prognosis of hepatocellular carcinoma. **Methods:** We conducted a prospective study of 191 patients diagnosed with HCC at Zhongshan Hospital from April 2016 to July 2016 as well as 99 healthy people. The platelet activation status was assessed by two platelet markers, PAC-1 and CD62p, using flow cytometry. The patients were treated with TACE or resection and monitored for ≥6 months. The diagnostic value of marker-positive platelets was determined by the receiver operating characteristic curve and the postoperative value were analyzed using the Kaplan-Meier method and COX regression model. **Results:** All the three groups with high levels of marker-positive platelets were likely to be diagnosed with HCC and the PAC-1+ percentage had the best efficacy. The univariate analysis showed that the levels of PAC-1+ and CD62p+ platelets was risk factors for poor postoperative prognosis after both TACE and resection. Moreover, the multivariate analysis revealed that the level of PAC-1+ platelets was an independent risk factor for poor prognosis. **Conclusion:** The PAC-1+ percentage of platelets is a new indicator for diagnosis and predicting postoperative prognosis.

A-348

Accuracy of Diagnosis and Genetic Profile of Breast Cancer in Sicily

C. Belgiovine[1], L. Paravizzini[2], L. Gullotti[2], G. Falzone[2], F. Zangrandi[1], M. Monari[1]. [1]*Humanitas Research Hospital, Pieve Emanuele, Italy,* [2]*Humanitas Centro Catanese di Oncologia, Catania, Italy*

Background: Breast cancer is the most common tumor in female; only in 2017 about 52.300 women fell it in Italy. This is a multifactorial pathology with a multifactorial ethology; breast cancer is a sporadic disease in the majority of the cases and it does not any kind of hereditary genes transmission, but in some cases, around 10% it could be hereditary and we could be studied the genetic transmission of genes, in particular BRCA1 -2, p53, PTEN, STK11, CDH1, PALB2, CHEK2, BARD1, BRIP1, NBN etc. Only in Sicily, 3.027 new cases every years are registered for this tumour with an incidence of 117 cases on 100.000 women and the two cities with the highest rate of this illness are Catania and Caltanissetta.

Methods: After diagnosis, performed by histological analysis, the patients meet the Oncologica Genetic Counselling (OGC), where the oncologist and geneticist analyzed the clinical history of the patients and, on the bases of literature guidelines, decide if do or not the next generation sequencing (NGS) test, a second level test, before therapy. In 5 months the OGC of Hospital Centro Catanese di Oncologia (CCO), decided to performed 48 NGS test in men and women

Results: Between 48 patients suspected for a hereditary breast cancer, 2 are men and 46 women with a median age of 44, 08 (26-75 years). At the light of these result we have registered 75% of positive cases between men (37 years old and 65 years old both BRCA2+) and 27, 27% between women (30, 5 median age for BRCA1+, 48, 01 median age for BRCA2+, 39 years old for family gene note mutation, and 34 years old for CHEK2).The total positivity for the test is 29, 16% and is very high compared to the Italian and world incidence.

Conclusion: These results supported and confirmed the optimal skimming operated by physician and could help our EUSOMA (European Society of Breast Cancer Specialist) unit to understand the genetic bases of high rate of breast cancer in Sicily to ameliorate direct screening and treatment. Applying quality indicators is essential to improve organization, performance and outcome in breast care. Efficacy and compliance have to be constantly monitored to evaluate the quality of patient care and to allow appropriate corrective actions leading to improvements in patient care, the goal of our work.

A-349

A Novel Mass Spectrometry Method for Monoclonal Free Light Chain Detection

S. North[1], J. Ashby[1], F. A. Sharpley[2], R. Manwani[2], S. Mahmood[2], S. Sachchithanantham[2], H. Lachmann[2], J. Gilmore[2], C. Whelan[2], P. Hawkins[2], S. Harding[1], A. Wechalekar[2]. [1]*The Binding Site, Birmingham, United Kingdom,* [2]*National Amyloidosis Centre, Royal Free Hospital, London, United Kingdom*

Background: The serum free light chain test (Freelite) is the only serum free light chain assay included in international guidelines for the identification, monitoring and response assessment of AL Amyloidosis. The assay relies upon perturbation of the free light chain (FLC) kappa / lambda ratio. Here we present a method using the serum free light chain antisera for identifying serum free light chain utilising Quantitative Immuno-Precipitation mass spectrometry (FLC-QIP-MS) and assess this method's sensitivity in previously diagnosed AL amyloidosis patient sera. **Methods:** Diagnostic sera from 18 biopsy positive amyloidosis patients (17 AL amyloidosis (14 λ, 3 κ), 1 equivocal amyloidosis) were available; for 2 patients matched sera were available at serum complete remission (but whom remained bone marrow minimal residual disease (MRD) positive). Para magnetic microparticles were covalently coated with modified polyclonal sheep antibodies monospecific for κ and λ FLCs. The microparticles were incubated with patient sera, washed and treated with acetic acid (5% v/v) containing TCEP (20 mM) to elute FLC in monomeric form (Tecan, Switzerland). Mass spectra were acquired on a Microflex LT/SH smart matrix-assisted laser desorption ionization time-of-flight mass spectrometer. FLC-QIP-MS results were compared to serum protein electrophoresis (Sebia, France) and Freelite turbidometric immunoassays (Optilite, The Binding Site Group Ltd., UK). **Results:** 100% of diagnostic samples were identified by an abnormal FLC ratio; the median involved κFLC was 78(73-440) mg/L and λFLC 185(44-1023) mg/L; with difference involved-to-uninvolved (dFLC) 118(33-1015) mg/L. 44% of samples were negative by all forms of serum electrophoresis, 17% were positive by immunofixation (IFE) only and 33% positive by serum protein electrophoresis (median(range) 11(1-17) g/L). The FLC-QIP-MS

assay confirmed normal polyclonal κ and λ FLC expression in the 17 control patients, and correctly identified the presence and type of monoclonal FLC in 3/3(100%) κ and 14/14(100%) λ patients (median mass-to-charge (m/z) ratio 11743(11687-11747) and 11334(11256-11706), respectively; p=0.014). In 1 equivocal amyloid patient whose amyloid fibril type remained unclear by immunohistochemistry, FLC-QIP-MS identified polyclonal κ and λ expression with an absence of monoclonal FLC; suggesting the amyloid is not related to light chain production. FLC-QIP-MS identified monoclonal λFLC expression in the two λ patients with samples at diagnosis and following achieving a serological CR post-treatment, with the same molecular mass in each case (m/z 11316 and 11653) at diagnosis and during CR; this was in the setting of normal λFLC levels (21.5 and 17.1 mg/L, respectively) and negative serum and urine IFE, but positive MRD. **Conclusion:** FLC-QIP-MS can accurately and sensitively detect monoclonal FLC and be concordant to the tissue amyloid type in AL amyloidosis. FLC-MS shows no evidence of contaminants interfering with the FLC, allowing small monoclonal FLC peaks to be identified in the presence of high polyclonal FLC background and/or within the normal range. FLC-QIP-MS is run in a robotic system opening the possibility of exploiting this technique for high-throughput analysis of amyloidogenic FLC.

A-350

Hotspot Mutation Detection in Pediatric Tumor Specimens with Custom Next Generation Sequencing Assay for the KEYNOTE-051 Study

B. Wei, D. Levitan, L. Chen, A. L. Webber, S. J. Diede. *Merck & Co., Inc., Kenilworth, NJ*

Background: The immune checkpoint inhibitor pembrolizumab is a humanized monoclonal antibody targeting PD-1 and has shown promising antitumor activity in adults with advanced solid tumors. The KEYNOTE-051 study evaluates pembrolizumab monotherapy for pediatric patients from 6 months to less than 18 years old with advanced melanoma or PD-L1 positive advanced, relapsed or refractory solid tumor or lymphoma. One of the study's secondary objectives is to assess mutations in *BRAF*, *KIT*, *NRAS*, *RB1*, *TP53*, *MAP2K1*, *PDGFA*, and *PDGFB* in tissue samples at baseline and in available samples at the time of progression.

Methods: To analyze hotspot mutations in the genes listed in the study objective, a custom next generation sequencing (NGS) assay was developed on the Ion AmpliSeq platform, an amplicon-based target enrichment technology often utilized for specimens with limited material. The assay was designed for degraded DNA extracted from formalin-fixed paraffin-embedded (FFPE) tissues. We modified the Roche High Pure FFPET RNA Isolation kit to maximize DNA extracted from the often limited pediatric tumor specimens. We utilized the NEBNext FFPE DNA Repair Mix to reduce cytosine deamination caused C>T|G>A artifact. The assay was validated with 35 commercial pediatric tumor specimens, with 2 to 3 specimens per tumor type, 2 commercial reference materials with multiple known mutations and 9 HapMap DNA samples, with confirmation from the FoundationOne NGS Panel or digital PCR for low frequency mutations (3-10%). The variant allele frequency (VAF) cutoff for the non-C>T|G>A calls is established at 3% and for the C>T|G>A calls 5%; any sample with VAF<10% is repeated with the NGS analysis to confirm the mutation calls. The NGS analysis was successfully performed on the 123 solid tumor specimens from the KEYNOTE-051 study, except for 4 with DNA yields below 20 ng and thus not analyzed. Only clinically relevant hotspot mutations, such as the ones recorded in NCBI ClinVar with clinical significance of pathogenic or likely pathogenic, are reported.

Results: 34 of the 119 analyzed pediatric tumor samples had at least 1 clinically relevant mutation detected by the custom NGS assay. 21 of the 34 samples with clinically relevant mutations had at least 1 *TP53* mutation. Some tumor types had high percentages of samples with mutations detected by the custom NGS assay. For example, 7 of 8 melanoma samples had either 1 *BRAF* or 1 *NRAS* mutation, with the detected variants known to be frequently mutated in melanoma. In addition, 3 of 5 melanoma samples with *NRAS* mutations had 1 additional *TP53* mutation. 9 of 12 glioblastoma samples had clinically relevant mutations, and 8 of them had *TP53* mutations. 1 *TP53* glioblastoma sample had 1 additional *BRAF* mutation, and 2 *TP53* glioblastoma samples had 1 additional *RB1* mutation. 2 glioblastoma samples had at least two *TP53* mutations.

Conclusion: A robust and sensitive NGS assay combined with efficient FFPE DNA extraction and repair methods was developed for pediatric tumor specimens. The assay has been successfully utilized to study mutations for potential association with pediatric tumors and/or treatment response to pembrolizumab.

A-351

Role of Biomarkers and Their Relation with Histological and Immunohistochemical Types in the Diagnosis of Breast Cancer in Young Women

G. M. R. Souza, A. C. J. Paniza, D. R. Ramadan, S. S. Andrade, S. Tufik, M. C. Feres. *Associacao Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil*

Background: Breast cancer is the most common malignant neoplasm in women with the highest associated mortality rate. Rapid screening programs can provide early diagnosis and increase the chances of survival. To determine prognosis and therapeutic options, it is mandatory to determine the histological grade and immunohistochemical profile of breast carcinomas with a panel composed of estrogen receptor (ER), progesterone receptor (PR), Ki-67 status and HER2 (epidermal growth factor human 2). There are no tumor biomarkers specific for the initial phase of the disease, however there are some already recognized that are required for monitoring of breast cancer, such as CA15.3, CEA and CEA125. The aim of this study was to correlate the histological types of breast cancer, immunohistochemical profile and conventional serum biomarkers in order to establish a greater impact on the early and noninvasive diagnosis of breast cancer. **Methods:** The present study consisted of an analytical and retrospective review of 714 patients who underwent breast biopsy between the years 2015 and 2017, with inclusion criteria: patients with a cut age up to 40 years old at the time of the test, positive result for breast cancer in the associated immunohistochemical panel biopsy. It was used as exclusion criteria: women aged over 40 years old, negative diagnosis for breast cancer in the biopsy, diagnosis for carcinoma in situ, absence of associated immunohistochemical panel. The data were obtained from the system of a large laboratory, which provides services to the public health system (sampling for convenience), which covers 70% of the health units in the State of São Paulo. **Results:** A total of 255 women were analyzed. Significant differences were found in patients with higher levels of the conventional markers CA15.3 and CEA. However, when commercial cut-off values were used, only CA15.3 was significant (p = <0.001). In the group of biomarkers, significantly higher levels were found. Using ANOVA, Post-HOC (Tukey's test) and CA15.3 sensitivity analysis methodologies, a correct diagnostic probability of 91.8% of breast cancer was found. When the altered CA15.3 values were compared with breast carcinomas, invasive carcinoma with lobular characters was the most histological type with the highest number of cases (p = 0.002). The results of immunohistochemistry for hormone receptors and HER2, being they divided in HR+, HR-, HER2+ and TN (triple negative) were also compared with the biomarkers, but there was no significant relation (p = 0.986). Of the analyzed results, 56.8% presented immunohistochemical profile HR+/ HER2- (n = 145); 22.7% presented a triple negative profile (n = 58). **Conclusions:** The model that combines the biomarker CA15.3 dosage in the evaluation of the suspicion of breast cancer should be considered since the results shows it may indicate carcinomas of lobular origin. New studies with greater sampling are necessary to verify the results obtained and to analyze other relationships and thus to increase the knowledge about this type of cancer, in order to propose more effective diagnostic and therapeutic methods.

A-352

Comparability and Precision of Four Oncological Methods on Seven Immunochemistry Analysers

P. Findeisen[1], I. Zahn[1], M. T. Haro Romero[2], D. Krempel[1], C. Garcia Rabaneda[2], T. De Haro-Muñoz[2], M. Barral Juez[2], K. Klopprogge[3], S. Engelmann[3]. [1]*Laboratory Dr. Limbach and Colleagues, Heidelberg, Germany,* [2]*UGC Análisis Clínicos intercentros, Hospital Universitario Campus de la Salud, Granada, Spain,* [3]*Roche Diagnostics GmbH, Mannheim, Germany*

Background: In our study we evaluated precision and comparability of four oncological markers (CEA, CA 15-3, fPSA and tPSA) evaluated at two sites in Europe. The analyses were run on seven commercially available immunochemistry analysers.

Methods: The data were evaluated in Germany and Spain and included the following immunochemistry analysers: two **cobas e** 801 analytical modules, one **cobas e** 601 system (Roche Diagnostics GmbH), ARCHITECT i2000SR (Abbott), UniCel DxI 800 (Beckman Coulter), Liaison® XL (DiaSorin), ADVIA Centaur XPT and IMMULITE 2000 XPi (Siemens Healthineers). For determination of the intralaboratory precision, pooled quality control material from Bio-Rad at three different concentration levels per analyte were distributed to both sites. Testing was done on five days in 5-fold determinations per assay using the pooled sample material. Coefficients of variation (CVs) were calculated per site as repeatability and within-lab precision. For method comparisons, aliquots of pooled native samples covering a broad concentration range per analyte were distributed to both testing sites and measured on the respective instruments.

Results: For the precision, tested with Bio-Rad control materials, the following intermediate precision CV ranges were calculated: CA 15-3 (~21.2 U/mL - 92.8 U/mL) 1.6% CV on the **cobas e** 801 system to 12.8% CV on ADVIA Centaur XPT; CEA (~ 3.0 ng/mL - 81.9 ng/mL) 0.9% CV on the **cobas e** 801 analyser to 7.0% CV on UniCel DxI 800; fPSA (~0.04 ng/mL -11.1 ng/mL) 0.8% CV on the **cobas e** 801 system to 9.6% CV on ADVIA Centaur XPT; tPSA (~0.08 ng/mL -31.1 ng/mL) 0.9% CV on the **cobas e** 801 analyser to 13.4% CV on ADVIA Centaur XPT. Elecsys systems showed a very good comparability of results using the same method: Passing/Bablok regression: slope 1.04 (fPSA) - 1.07 (CEA), Pearson's r correlation 0.9993 - 0.9998. The comparability to other tested methods was shown with Passing/Bablok regression slopes ranging from 0.40 (CA 15-3, UniCel DxI 800) to 1.33 (CEA, UniCel DxI 800), Pearson's r correlation 0.9504 (CA 15-3, UniCel DxI 800) to 0.9989 (tPSA, ADVIA Centaur XPT and IMMULITE 2000 XPi).

Conclusion: The data of our study support laboratories in assessing the precision and comparability of their routine oncology methods.

A-353

Additional Actionable Mutations in Primary Colon and Lung Adenocarcinomas Identified by Next Generation Sequencing in South Florida Veteran Population

S. M. Mastorides, C. P. Wilson, G. T. Carlton, N. Ashraf, L. B. Thomas, L. A. Deland, A. A. Borkowski. *James A. Haley VA Hospital, Tampa, FL*

Background: Molecular profile testing of tumor tissue is a growing and vital need in the treatment of patients with colon cancer and non-small cell lung cancer. According to the National Comprehensive Cancer Network (NCCN) guidelines, routine molecular testing is recommended to identify rare driver mutations that may be present in tumor tissues. Proven responses to molecular therapies have greatly increased the improvement of care in colon and lung cancer patients. Currently, NCCN guidelines for colon adenocarcinomas suggest testing for tumor mismatch repair or microsatellite instability status and determination of tumor gene status for RAS (KRAS and NRAS) and BRAF. The current NCCN guidelines for adenocarcinomas in non-small cell lung carcinomas include testing for EGFR, ALK, ROS1, BRAF mutations and PD-L1 expression. Present test methods for both sets of guidelines include FISH, PCR based sequencing, and immunohistochemistry. The objective of the study was to identify additional actionable markers from colon and lung adenocarcinomas, through next generation sequencing, that would not be detected based on current test methodology. **Methods:** Fifty-eight cancer cases were reviewed and selected from both male and female adenocarcinoma patients for next generation sequence testing. Samples were sent to Personal Genome Diagnostics (Baltimore, MD) for Cancer Select 125 testing. Next generation sequence testing was performed by analyzing the coding regions of 125 genes and identifying tumor-specific alterations in categories including: microsatellite instability, sequence mutations, amplifications, and rearrangements. The annotated reports included detailed analysis of mutations detected, FDA-approved therapies (for same and other indication) and current clinical trials. Actionable markers were identified and categorized based on primary tumor location, then further subcategorized into sequence mutations, amplifications and rearrangements. **Results:** Actionable markers detected by next generation sequencing were divided based on colon versus lung primary tumors. Results were accumulated over a nineteen-month testing period. Current molecular markers tested, according to NCCN guidelines, were removed from the data set leaving only the additional actionable markers. Microsatellite instability screening markers, KRAS, BRAF, and NRAS mutations were removed from the colon adenocarcinoma category. EGFR mutations, BRAF mutations, ALK rearrangements, and ROS1 rearrangements were removed from the lung adenocarcinoma category. The colon primary tumor category (N=30 patients) yielded 18 additional actionable sequence mutations, 16 amplifications and 5 Bethesda microsatellite markers. The lung primary tumor category (N = 28 patients) yielded 19 additional sequence mutations, 16 amplifications and 1 rearrangement. **Conclusion:** Next generation sequencing of primary colon and lung adenocarcinomas provides accurate and comprehensive data detailing actionable gene alterations within a tumor sample. Tumor molecular alterations are listed in categories including: consequence of sequence mutation, exon location, mutant fractions, and fold increases for amplifications. Gene alterations are further designated by current FDA approved therapy (for same and other indications) and current clinical trials. Our study identified several additional actionable markers within each sample that would not have been discovered utilizing our current test methods. Based on our findings, we conclude that next generation sequencing is an accurate and powerful test method that should be performed whenever adequate tumor tissue is available.

A-354

Serum Dosage of p2PSA and Correlation with the ISUP Classification for Prostate Cancer - A Retrospective Study

A. C. J. Paniza, M. C. Feres, F. S. Siqueira, G. M. Souza, B. B. Perez, S. S. Andrade, C. S. Silva, M. C. De Martino, D. R. Ramadan, S. Tufik. *Associacao Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil*

Background: Prostatic specific antigen (PSA) testing in conjunction with digital rectal examination (DRE) is a screening tool in diagnosis of prostate cancer and can reduceup to 21% the risk of death of men with prostate cancer. The disadvantage of using total PSA (T-PSA) test for detection of prostate cancer is it's low specificity, since any local infection or prostate manipulation can raise its values. The indication for transrectal ultrasound with biopsy for the detection of prostate cancer is a T-PSA level ≥4 ng/mL or abnormal findings from a DRE. Unnecessary prostate biopsies can lead to overdiagnosis, overtreatment, and anxiety for the patient and high costs to the public health system. Recent studies have demonstrated a higher specificity of [-2] proPSA (p2PSA), a fraction of PSA that rises only in the presence of neoplasia. Based on the high prevalence of prostate cancer nowadays, it is necessary that more specific detection methods be tested and become more accessible, allowing a more accurate medical intervention. **Patients/Methods**: Were tested 165 blood samples of men, aged between 54 and 87 years, collected during the year of 2018 in a public health reference laboratory in São Paulo, Brazil. Serum samples were measured for the concentration of T-PSA, free PSA (F-PSA) and p2PSA with the Beckman Coulter Immunoassays in Access Hybritech. The prostate health index (PHI) was calculated. Of these samples, 30 cases with paired prostate biopsy were selected for statistical analysis. The biopsies were classified according to the International Urological Pathology Society (ISUP) prognostic groups as follows: group A, corresponding to grades 1 and 2 of ISUP and group B, corresponding to grades 3, 4 and 5 of ISUP. The Analysis of Variance ANOVA test was performed to verify differences between groups A and B in function of PSA values, with a Post-HOC test complementary. **Results:** The ANOVA test showed differences between the groups A and B according to the value of p2PSA with a value of p=0.037. The Post-HOC test showed that p2PSA can distinguish the grades 1 and 5 of ISUP (p=0.027) and an average of 20.1±16.9 in ISUP 1 group versus 239.4±0 in ISUP 5 group. The others dosages, PHI, T-PSA and F-PSA, were not statistically significant, with p value ranging from p=0.067 for PHI and p=0.549 for T-PSA. The sensitivity of p2PSA to include the patient in group B is 3% at each unit dosed (OR = 1.035), with an average sensitivity of 62%. The specificity of p2PSA was 87%. T-PSA showed an average sensitivity of 57% and a specificity of 75%. **Conclusion:** The current study could verify that the serum p2PSA dosage is a more sensitive and more specific test when compared to the T-PSA and F-PSA dosages. In addition, it presents a higher correlation with the ISUP prognostic groups for prostate cancer. Thus, this test can aid in the correct indication or proscription of prostate biopsy.

A-355

Cytogenetic Changes Present in Acute Promyelocytic Leukemia and the Significance in the Prognosis of the Patient

M. M. Almeida, J. M. Fenandes. *DASA, Barueri, Brazil*

Background: Acute promyelocytic leukemia (APL) or AML-M3 corresponds to 10 - 15% of cases of acute myeloid leukemia's (AML). The clinical course of the APL has been modified in recent years, an acute leukemia rapidly fatal to one of the most curable subtype of AML. About 90% of cases, is related to translocation t(15; 17)(q24; q21) and 30%- 40% of patients during the diagnosis had a chromosome abnormalities in addition to t(15; 17), in 2% of cases the variant translocations, when the RARα gene can be translocated to other chromosomes.

Objective: Verify the incidence of chromosomal abnormalities in addition to t(15;17) in APL patients.

Methods: Retrospective survey of G-band karyotypes performed by the laboratory during the year of 2018 with diagnostic hypothesis of APL.

Results: Of 35 samples analyzed with diagnostic hypothesis of LPA, during the year 2018, 11 cases presented normal karyotype, 2 cases had absence of mitotic metaphases and 22 cases with chromosomal abnormalities, being 21 cases of classic translocation t(15;17) and 1 case of a rare alternative translocation (t(11; 17)(q23; q21)-ZBTB16/RARE). It was observed the presence of additional chromosome in 19% of cases t(15; 17), the most common being the trisomy 8 (4/21 cases).

Conclusion: The most common associated chromosomal abnormality is trisomy of chromosome 8, which compared to literature, was expected. Recent reports don`t present difference in prognosis of patients with additional changes to that feature only the t(15;17)(q24; q21). The few cases with translocations alternatives described in the

literature had a survival similar to those with simple translocation, with the exception of patients with ZBTB16/RARE and STAT5B/RARE - der(17) are resistant to ATRA and has a bad prognosis.

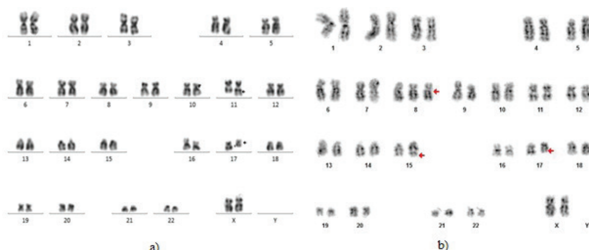


Fig 1. a) G-Brand karyotype with rare alternative translocation – t(11; 17)(q23; q21)-ZBTB16/ RARE.
b) G-Brand karyotype with translocation and trisomy of chromosome 8 – t (15; 17)(q24; q21),+8.

A-356

Method Comparison and Reference Limits for Two Chromogranin A (CgA) Assays

J. A. Erickson[1], F. Chiang[2], C. M. Walker[2], J. R. Genzen[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT*

Background: Chromogranin A (CgA) is a 49 kD prohormone found in the secretory granules of neuroendocrine cells. The protein contains several recognition sites for endopeptidases that cleave the protein into several functional peptides. Among these are vasostatin I and II, pancreastatin, catestatin, and prochromacin. As a marker for functional and non-functional neuroendocrine tumors, studies have shown serum CgA concentrations to be useful clinically for the detection and monitoring of disease. Here we describe a method comparison between a CgA micro-titer plate ELISA (Chromogranin A ELISA kit, Cisbio Bioassays) and an analyzer based assay (B·R·A·H·M·S CgA II KRYPTOR, Thermo Fisher Scientific, Inc). Reference limits for each assay were also established.

Methods: Specimens were collected according to Institutional Review Board approved protocols. Serum CgA was measured according to each assay manufacturer's testing protocol. A method comparison was conducted utilizing 149 specimens. Reference limits were established for each assay by testing the same cohort of 125 healthy adult volunteers, ages 19 - 65 yrs old. Data analysis was performed using Analyse-it Method Validation Edition (version 4.65, Analyse-it Software, Ltd.) and Prism (Prism 5, GraphPad Software).

Results: Deming regression of the KRYPTIOR vs. the Cisbio CgA assays generated a slope of 0.695 and y-intercept of 7.215, with an $r^2 = 0.947$. Non-parametric reference limits at the 95th percentile were determined to be 160 and 103 ng/mL CgA for the Cisbio and KRYPTOR assays, respectively. No significant differences were identified between genders, with p-values of 0.834 and 0.355 for the Cisbio and KRYPTOR assays, respectively. CgA concentrations increased slightly but significantly with age (Cisbio: $r^2 = 0.095$, p = 0.0006. KRYPTOR: $r^2 = 0.111$, p = 0.0002) but the increases were deemed clinically insignificant.

Conclusions: An adequate correlation is observed between the Cisbio Bioassays Chromogranin A ELISA and the B·R·A·H·M·S CgA II KRYPTOR assay. However, there is significant disagreement overall. As a result, reference limits cannot be used interchangeably.

A-357

Novel Circulating Protein Biomarkers for Thyroid Cancer Revealed using Orbitrap Q-Exactive-plus

D. Li[1], W. Cheng[2], J. Wu[1], Z. Liu[1], C. Ma[1], Y. Zou[1], D. Wang[1], X. Guo[1], C. An[3], Y. Zhang[2], L. Qiu[1]. *[1]Department of Clinical Laboratory, Peking Union Medical College Hospital, Peking Union Medical Colle, Beijing, China, [2]Department of Clinical Laboratory, Zhejiang Cancer Hospital, Hangzhou, Zhejiang, China, Zhejiang, China, [3]Department of Head and Neck Surgery, National Cancer Center/National Clinical Research Center for Cancer/ Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, 100021, China, Beijing, China*

Background: Distinguishing different types of thyroid cancers is still challenging in clinical laboratories. As different types of tumors require completely different interventions in the clinic, it is necessary to establish new methods for accurate diagnosis of thyroid cancer.

Methods: The proteomics of human serum was screened using Orbitrap Q-Exactive-plus among 31 patients with thyroid cancer (TC) (stages I-IV) including 15 cases of papillary thyroid cancer (PTC), 10 medullary thyroid cancer (MTC), and 6 follicular thyroid cancer (FTC) along with 15 patients with benign thyroid nodules (TN) and 15 healthy controls (HC). Subsequently, 17 differential proteins were verified in 291 patients with TC (stages I-IV) including 247 papillary thyroid cancers, 38 MTCs, and 6 FTCs along with 69 patients with benign TN and 176 HCs using enzyme-linked immunosorbent assay.

Results: A total of 519 proteins were detected in serum samples using Orbitrap Q-Exactive-plus. Amyloid beta A4 protein, apolipoprotein A-IV, gelsolin, contactin-1, gamma-glutamyl hydrolase, and CFHR1 were chosen for further validation. The median serum values of CFHR1 were significantly higher among MTC and FTC groups when compared to those of PTC and control groups ($P < 0.001$). CHFR1 exhibited higher diagnostic performance in distinguishing patients with MTC from those having PTC ($P < 0.001$) with sensitivity 100.0%, specificity 85.08%, area under the curve 0.93, and cut-off of 0.9185 ng/ml.

Conclusion: Our study illustrates that the Orbitrap Q-Exactive-plus was useful in investigating possible serum biomarkers for TCs. CFHR1 might serve as a novel biomarker to distinguish PTC from MTC with high sensitivity and specificity.

A-358

N Latex FLC Kappa and Lambda Assays for Monitoring Multiple Myeloma: Method Comparison Study versus FREELITE Assays

W. Gentzer[1], M. Goginko[2], R. Christenson[3], K. Lockington[4], A. Mohammad[2], A. Peter[5], M. Ruiz[3], J. Troynacki[6], K. Weisel[5], M. Willrich[4], L. Wagner[1], C. Wagner[1]. *[1]Siemens Healthcare Diagnostics Marburg GmbH, Marburg, Germany, [2]Baylor Scott&White Health Center, Temple, TX, [3]University of Maryland School of Medicine, Baltimore, MD, [4]Mayo Clinic, Rochester, MN, [5]University Hospital Tübingen, Tuebingen, Germany, [6]Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: Free light chain (FLC) kappa and lambda testing is part of the recommended test panel for therapy monitoring in light chain multiple myeloma (LCMM) and is frequently applied as an additional marker in multiple myeloma (MM). **Methods:** In a prospective multicentric method comparison study, FLC testing using Siemens Healthineers' N Latex FLC kappa and lambda assays (N Latex FLC) and The Binding Site's FREELITE kappa and lambda assays (FREELITE) were compared with respect to the therapy response criteria obtained according to the IMWG MM guidelines. For therapy response evaluation, the difference of involved to noninvolved FLC was considered for therapy response classification, as well as the FLC ratio for the definition of stringent complete response. Response status of each monitoring event was categorized into six response categories—stringent complete response (sCR), complete response (CR), very good partial response (VGPR), partial response (PR), stable disease (SD), or progressive disease (PD)—by applying the two different FLC assays. The response categories obtained by the two assays were compared. A total of 98 patients (17 LCMM and 81 non-LCMM) with 391 follow-up blood draws (at least four draws per patient in intervals ≥3 weeks) were included. **Results:** Total agreement rate of the classification based on applying either N Latex FLC or FREELITE assay results in the evaluation algorithm was 83.9% (Cohen's kappa coefficient: 0.70). For LCMM, an agreement of 77.3% was obtained (Cohen's kappa coefficient: 0.69). For MM, a substantial 85.2% agreement rate (Cohen's kappa coefficient: 0.69) was obtained. The majority of draws with discordant response evaluation were VGPR/PR (22%) and PR/SD (33%). When comparing the response categories obtained by the two assays with the clinical status provided by the treating physician,

the total agreement rate was identical, with 54% for both the N Latex FLC and FREELITE assays. This low concordance rate was not unexpected, as a major advantage of FLC testing is its fast response to a change in disease activity. **Conclusion:** In this study, the N Latex FLC and FREELITE assays generated a good agreement rate for the response classification obtained by application of either test system; both assays showed a similar, relatively low agreement rate versus the clinical status assessment, which incorporates much more information, both clinical and laboratory, than serum marker testing alone.

A-359

Performance of Improved RT-qPCR Assays to Accurately Monitor BCR-ABL1 Major Fusion Transcripts in Chronic Myeloid Leukemia (CML): Transition from MR4.5 to 4.7

D. C. Torres[1], C. P. F. N. Machado[2], D. Zauli[1]. [1]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil,* [2]*Hermes Pardini Institute (Progenética Laboratory), Belo Horizonte, Brazil*

Background: Monitoring of BCR-ABL1 fusion transcripts is decisive for clinical assessment of CML patients treated with tyrosine kinase inhibitors (TKIs). Quantitation of the standardized international scale (IS) percentage of BCR-ABL1 transcripts against a common baseline by RT-qPCR has become the gold standard strategy that defines molecular response (MR) in CML. Assessment of major molecular response (MMR; MR>3.0) is associated with favorable survival outcome. Currently, there are several validated commercial kits available for measuring BCR-ABL1 from different companies, in addition to new and optimized products developed from the same manufacturer. Herein, our goal was to evaluate the performance of current Asuragen's FDA-cleared QuantideX® qPCR BCR-ABL IS Kit, which identifies major (e13a2, e14a2) fusions, in comparison with its previous clinically validated BCR/ABL1 Quant Test Kit, which targets both major and minor (e1a2) transcripts.

Methods: RNA samples from seventy-five clinical specimens were assessed in parallel with Asuragen's BCR/ABL1 Quant Test (Asu-2bcr) and QuantideX qPCR BCR-ABL IS Kit (Asu-Mbcr). According to manufacturer's manual, Asu-Mbcr precision was tested up to MR3.7, thus we performed five replicates for 12 samples from BCR-ABL1 values ranging from MR1.0 to MR4.7. RT-qPCR was run in singlet on an ABI 7500 Fast instrument, software version 2.3, following manufacturer's protocol (Asuragen). Statistical analyses were performed with GraphPad Prism 7.0.

Results: Asu-2bcr analysis showed 34 positive and 41 negative samples. There were two samples containing e1a2 transcript, 13 e13a2, 9 e14a2, and 10 e13a2/e14a2. Asu-Mbcr analytical specificity was supported by negative amplification of e1a2 samples and positive of all major transcripts. Precision analysis of Asu-Mbcr showed high correlation among replicates (Pearson R coefficient > 0.99) across the dynamic range (MR1.0 to MR4.7), supporting singleton testing as claimed in the Instruction for Use. MR standard deviation among replicates increased from ~ 0.03 (MR1.0 and 2.0) to 0.1-0.16 at MR>3.0. The correlation of positive MR values between methods was excellent, with a Pearson R correlation coefficient of 0.96. The concordance for MMR was 94%. MR differences between kits were 0.26 ($MR^{1.0}$), 0.24 ($MR^{2.0}$), 0.33 ($MR^{3.0}$), and 0.32 ($MR^{4.0}$). Although, there is a tendency to higher differences at MR>3.0, it was not statistically significant. Quantitative results were not reproducible across all specimens: twelve negative samples (29%) from Asu-2bcr became positive with Asu-Mbcr (>MR3). The results suggest a higher analytical sensitivity of the Asu-Mbcr test (LOD of Asu-Mbcr is MR4.7 or 0.002%IS, while Asu-2bcr is MR4.5 or 0.0032%IS). Indeed, 67% of false negative samples from Asu-2bcr had MR>4.1 with Asu-Mbcr. In addition, 84% of positive samples in both methods had slight lower MR values when tested with Asu-Mbcr.

Conclusion: These findings suggest that Asu-Mbcr is indeed a more robust and sensitive method than Asu-2bcr. Small MR differences might be observed during transition between kits and should be addressed carefully over validation in diagnostic laboratories.

A-360

Molecular Monitoring of p190$^{BCR\text{-}ABL1}$ Levels should not be Neglected in Chronic Myeloid Leukemia (CML) Patients Treated with Tyrosin Kinase Inibitors (TKIs)

D. C. Torres[1], F. K. Marques[1], F. L. O. Marinho[1], C. P. F. N. Machado[2], M. K. Lima[2], D. Zauli[1]. [1]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil,* [2]*Hermes Pardini Institute (Progenética Laboratory), Belo Horizonte, Brazil*

Background: The majority of CML patients (~95%) expresses the p210 BCR-ABL1 fusion transcript (e13a2, e14a2), while a few CML cases (1-2%) have the p190 transcript (e1a2). Detection of e1a2 transcripts is associated with worst response to TKIs and rapid disease progression. p190 may also be co-expressed with p210, usually due to alternative or mis-splicing. The aim of this study was to determine the frequency of transcript variants (p190 and p210) among Brazilian patients from Hermes Pardini Institute and evaluate p190 emergence over quantitative monitoring of BCR-ABL1 during TKI treatment.

Methods: From January 2015 to December 2018, p210 and p190 trancripts were evaluated at diagnosis from RNA clinical samples (EDTA-peripheral blood or bone marrow) by multiplex RT-PCR, following BIOMED-1 guidelines. Then, BCR-ABL1 transcript levels were monitored every 3 months from CML patients treated with TKIs. Quantitation of the standardized international scale (IS) percentage of BCR-ABL1 transcripts against a common baseline was assessed by RT-qPCR with Asuragen's BCR/ABL1 Quant Test. Samples that showed an emergence of e1a2 expression over time were catalogued and analyzed for the present study.

Results: A total of 354 CML samples were examined at diagnosis. The majority had transcripts with e14a2 (42.9%) or e13a2 (36.4%) junctions, while 2.5% had e1a2. Higher frequencies of e14a2 have been observed in European, Indian, and Brazilian populations, but not in Ecuador, Mexico, and Venezuela, for instance. In 17.8% of the cases, both e13a2 and e14a2 transcripts were co-expressed and there was one case with e14a2/e1a2 (0.3%). In this study, a high frequency of e13a2/e14a2 was detected in comparison to internationally reported cases (usually < 10%). This might be explained by Brazil's continental dimension together with a heterogeneous genetic background from African, Amerindian, Asian, and Caucasian populations. No significant differences ($P > 0.05$) were observed between women (n=163) and men (n=191) among the different fusion transcripts, but CML in general was significantly more frequent in male patients ($P = 0.01$; odds ratio of 1.34) than BCR-ABL1 negative individuals. Patients' age range and mean (±SD) were 6 to 95 and 56.47±15.41 years old, respectively. There were no significant age differences between p210 positive and negative groups ($P > 0.05$). Nevertheless, CML patients expressing e1a2 were significantly older ($P = 0.02$; 68±5.3 years) than negative ones. RT-qPCR analysis showed emergence of co-expression of p210 and p190 in seven cases previously determined as p210 only. The median of BCR-ABL1 ratio was 0.0372% ranging from 0.0085 to 2.858%. Hence, suggesting a tendency of p190 emergence in p210 positive samples at lower BCR-ABL1 ratios. Moreover, three p210 cases relapsed after 8, 43, and 90 months of complete molecular remission (undetected BCR-ABL1 copies), all expressing p190.

Conclusion: Identification of the specific fusion transcript in CML patients is important due to the difference in prognosis and response to therapy. The present data reinforces that p190 may happen as a secondary event in some cases and, thus, should be closely monitored after diagnosis due to the high incidence of disease progression and treatment resistance/failure.

A-361

Validation of Interphase Fluorescence in Situ Hybridization (FISH) Panel for Detection of the Chromosomal Abnormalities in Patients with Multiple Myeloma

F. K. Marques, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Multiple myeloma (MM) is a malignant neoplasm of plasma cells (PC) that accumulate in bone marrow, leading to bone destruction and marrow failure. Newly diagnosed MM is sensitive to a variety of cytotoxic drugs. Although response are durable, relapse is an expected part of the disease course. MM is not considered curable with current approaches. Studies of the associated cytogenetic abnormalities indicate that MM is a heterogeneous disease, suggesting that risk adapted approaches and individualizing treatment will help refine patient management. Bone marrow studies at initial diagnosis should include chromosome analysis by metaphase cytogenetic and FISH performed in PCs. Translocations, deletions and amplifications have been identified in patients with MM. Stratification of patients in risk group based on

the chromosomal abnormalities is being utilized for prognostic and selection therapy. The study of cytogenetic abnormalities by karyotyping is limited because of the low mitotic index of the malignant PCs. Interphase fluorescence in situ hybridization (FISH) does not depend on cell proliferation, but its detection sensitivity is limited by the percentage of PCs in whole bone marrow. It has been recommended that FISH should be performed in combination with PCs targeting strategies.

Objective: To validate an interphase fluorescence in situ hybridization (FISH) panel for MM using CD138 positive cells.

Methods: Ten bone marrow samples from patients diagnosed with MM received at the private Cytogenetic Laboratory in Brazil were used to standardize a panel of six probes [1p/1q deletion/amplification, 13q14 deletion, 17p deletion (TP53), t(4;14)(FGFR3/IGH), t(11;14)(CCND1/IGH) and t(14;16)(IGH/MAF)] in $CD138^+$ PCs purified by magnetic cell sorting. One experienced cytogeneticist analyzed 100 $CD138^+$ PCs. The same 10 samples were subjected to 24 hour culture and 200 unsorted bone marrow cells were analyzed using the six probes by the same cytogeneticist. The results of the two analyzes were compared. The process started between 6 - 24 hours after the sample collected following the manufacturers' recommendation.

Results: $CD138^+$ PCs FISH identified more abnormal cases (10 vs. 6 cases) and more chromosome abnormalities than conventional FISH. Only one case had abnormalities found by both karyotyping and FISH. The most frequent abnormalities detected were amplification 1q, monosomy 13, extra copia of CCND1 (trisomy 11?) and t(11;14)(CCND1/IGH). An enhanced sensitivity of PC FISH was observed with all probes. The percentages of abnormal cells differed significantly between the results from two methods. The median percentages of abnormal cells detected by PC FISH and conventional FISH were 84% vs. 0.5% for amplification 1q.

Conclusion: FISH analysis has revolutionized genetic analysis and enabled a more precise determination of the presence and frequency of genetic abnormalities. Although only ten cases were used in this validation, a high rate of abnormalities was seen. An important issue is the cut-off levels for FISH probes. Some authors use statistical analysis of normal control cases to determine the cut-off levels. However, to select PCs from normal bone marrow is very difficult. The European myeloma network recommends a conservative cut-off: 10% for fusion and breakapart probes and 20% for numerical aberrations.

A-362

Tumor Mutation Burden (TMB) in FFPE Samples: A Pilot Study Using a Large Gene-target Sequencing Panel

M. A. Pereira, F. L. O. Marinho, R. L. M. Guedes, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Tumor Mutation Burden (TMB) is defined as the number of somatic mutations normalized by a coding area in a cancer genome and it has been recently discovered as an important quantitative biomarker for predicting the response for cancer immunotherapies. TMB and mutation types vary widely among different cancer types, but generally, high TMB is associated with increased neoantigens and better response to immune checkpoint inhibitors. Targeted NGS panels are robust, affordable and faster alternatives to Whole Exome Sequencing for estimating the patient's TMB, although its implementation into clinical routine is still a challenge given several preanalytical, analytical and postanalytical caveats. **Objective:** Measuring TMB in solid tumor samples using a large gene-targeted sequencing panel (NGS) for routine diagnostics implementation. **Methodology:** Tumoral DNA was extracted from 17 FFPE samples using RecoverAll™ Total Nucleic Acid Isolation (Thermo Fisher) and/or QIAamp DSP DNA Mini (QIAGEN) kits. 20ng or 30ng of DNA was used for TMB assessment by Thermo Fisher's Oncomine™ Tumor Mutation Load Assay (targeting ~1.7 Mb, 409 cancer-related genes) on Ion S5™ System. Signal processing, base calling and sequence alignment against the hg19/GRCh37 reference genome were performed on Torrent Suite™ 5.10 Server. Variant calling and TMB calculation were performed on Ion Reporter™ 5.10 software. TMB was measured by counting the nonsynonymous somatic mutations (SNVs and Indels) per Mb at >= 5% (default) and 10% allelic frequency. Five samples were also tested for microsatellite instability (MSI). **Results and Discussion:** Two samples were discarded due to low coverage mean depth (<300X) and library quality. TMB measurement did not show significant variation regardless extraction methods and DNA input ($p>0.05$). However, preanalytical factors had a strong influence for TMB assessment. A high baseline noise was observed in two samples due to cytosine deamination (C:G>T:A) which led to TMB overestimation (>2,000). Since C>T low allelic frequencies are common FFPE artifacts, 10% allelic frequencies were also used as a cutoff to count mutations. Nevertheless, TMB remained overrated. For the other samples, TMB values were: TMB<5 (n=5; 2 with low MSI), 5<TMB<10 (n=4) and TMB>20 (n=4; 3 with high MSI). **Conclusions and Perspectives:** Manual and automatic extraction methods showed similar results. However, artifactual alterations need to be minimized. Since cytosine deamination is the most prevalent sequence artifact in FFPE samples, uracil-DNA glycosylase treatment will be performed. Heat treatment and proteinase K extension is also being evaluated. After treatment choice, non-tumoral DNA samples will also be analyzed, helping to establish cancer-specific TMB cutoffs. An efficient TMB detection could avoid unnecessary immunotherapies improving treatment efficacy and saving costs.

A-363

Retrospective Analysis of FLT3-ITD and FLT3-TKD (D835) Mutations in a Brazilian Cohort

F. K. Marques[1], D. C. Torres[1], P. G. Silva[2], D. Zauli[1]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Molecular Genetics Division), Belo Horizonte, Brazil*

Background: Acute myeloid leukemia (AML) is a clonal disorder characterized by various genetic abnormalities and variable response to treatment. Mutations of the FMS-like tyrosine kinase 3 (FLT3) gene occur in approximately 30% of all AML cases. Activating mutations in FLT3 include internal tandem duplication (ITD) and point mutations in the tyrosine kinase domain, typically at the activation loop residue D835 (TKD). The FLT3-ITD is the most frequent mutation and it is associated with increased risk of treatment failure and poor prognosis. The prognostic value of FLT3-TKD mutation, which has a lower incidence in AML (approximately 7-10% of all cases), is uncertain. Despite the evident poor outcome associated with FLT3-ITD, it is unclear whether this can be due only to the presence or absence of the abnormality, or whether other factors such as mutant allele burden influence its prognostic impact. For instance, a high total mutant level (more than 50%) has revealed worse clinical outcome. **Objective:** To investigate the frequency of FLT3-ITD and FLT3-TKD (D835) mutations in clinical samples from a Brazilian private laboratory. **Methods:** We retrospectively studied FLT3-ITD and FLT3-TKD (D835) mutation outcome in AML patients at the Hermes Pardini Institute from January 2012 to December 2018. FLT3-TKD mutation was determined by PCR and gel electrophoresis, while FLT3-ITD was followed by capillary electrophoresis (ABI 3730). **Results:** A total of 226 samples were analyzed: 121 male and 105 female patients. Forty-five (19.9%) patients had FLT3 mutation: 37 FLT3-ITD (16.4%), 6 FLT3-TKD (2.7%), and 2 (0.8%) had both mutations. The median age of patients without FLT3 mutation was 44 years (1 - 83 years), and for the mutated group was 46 years (6 - 84 years). In addition, it was conducted a quantitative analysis of 16 ITD positive patients by calculating the signal ratio between the mutant and wild type alleles. The median allelic ratio of FLT3-ITD was 0.55 (ranging from 0.04 to 4.86). Cytogenetic analysis was performed in 13 FLT3 positive cases. Several abnormalities were observed, including: t(15;17) (n=2), t(2;9) (n=1), inv(3) and monosomy 7 (n=1), t(9;11) (n=1), and del(10) (n=1). Gene mutations other than FLT3 consisted of NPM1 in three cases. On the other hand, genetic alterations coexisting with FLT3 were NPM1 in two cases and CEBPA biallelic mutation in one. **Discussion and Conclusions:** FLT3 mutation analysis is capable of guiding clinical decision that potentially includes alternative treatments, such as FLT3 inhibitors. Recent guidelines have shown that quantitative FLT3-ITD allelic ratio and its correlation to other gene abnormalities and cytogenetic analysis is of extreme diagnostic importance. Currently, a favorable prognostic for AML can be assessed either by biallelic mutation of the CEBPA gene or by detection of NPM1 mutated without FLT3-ITD or even $FLT3\text{-}ITD^{low}$ mutation.

A-364

Long Noncoding RNA LINC02418 Regulates MELK by Acting as a ceRNA and may Serve as a Diagnostic Marker for Colorectal Cancer

C. Wang, Y. Zhao, L. Du. *Department of Clinical Laboratory, The Second Hospital of Shandong University, Jinan, China*

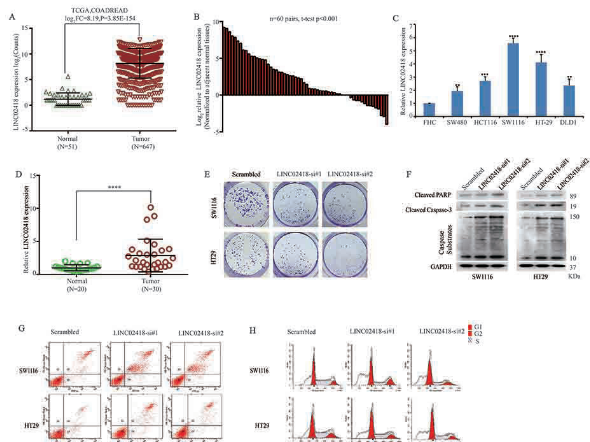
Background: Some types of long non-coding RNAs (lncRNAs) are aberrantly expressed in human diseases, including cancers. However, the overall biological roles and clinical significances of most lncRNAs in colorectal cancer (CRC) are not fully understood.

Methods: Firstly, TCGA was analyzed to identify the differentially expressed lncRNAs between CRC tissues and noncancerous tissues. Next, we evaluated the effect of LINC02418 on the CRC cells tumorigenesis, and its regulation of absorbing microRNA and indirectly stimulating protein expression by acting as a ceRNA. Further, the diagnostic performance of exosomal LINC02418 was evaluated by the receiver operating characteristic curve (ROC) and the area under curve (AUC).

Results: After using publicly available expression profiling data and integrating bioinformatics analyses (Fig.1A), we identified that LINC02418 was highly expressed in

CRC tissues, cell lines and peripheral serum (Fig.1B-D). Furthermore, LINC02418 silencing inhibited CRC cell proliferation (Fig.1E) by promoting apoptosis and inducing cell cycle arrest (Fig.1F-H). Mechanistically, LINC02418 acted as a ceRNA to upregulate MELK expression by absorbing miR-1273g-3p, and a positive correlation was also identified for the fold change between the LINC02418 mRNA level and MELK expression. In addition, exosomal LINC02418 could distinguish patients with CRC from healthy controls (AUC = 0.8997).

Conclusions: Collectively, we determined that LINC02418 was significantly overexpressed in CRC, and LINC02418-miR-1273g-3p-MELK axis played critical role in MELK tumorigenesis. Additionally, exosomal LINC02418 was a promising novel biomarker that could be used for the clinical diagnosis of CRC.



A-365

Impact of Using Sensitive LC-MSMS Based Testosterone Analysis for Prostate Cancer Patients; A Requirement for Testosterone Analysis in Castrated Men?

L. J. van Winden, M. Lanfermeijer, O. van Tellingen, A. Bergman, H. G. van der Poel, H. van Rossum. *Netherlands Cancer Institute, Amsterdam, Netherlands*

Background: Liquid chromatography tandem-mass spectrometry (LC-MSMS) assays are considered the best practice for steroid analysis. To address in-house clinical diagnostics, an LC-MSMS assay was developed for quantitation of cortisol, progesterone, androstenedione and testosterone. Specifically, we investigated the impact of this new assay on testosterone quantitation in prostate cancer patients to assess castration status. **Methods:** The LC-MSMS assay consisted of liquid-liquid extraction, reverse phase chromatography and MRM analysis on a QTRAP 6500+ (ABSciex). Standard validation experiments were performed based on Clinical and Laboratory Standards Institute (CLSI) guidelines. Finally, 134 testosterone requests from urologist and 101 requests from oncologist both treating prostate cancer patients were compared with Roche Cobas immunoassay (IA). **Results:** The obtained testosterone LLOQ was 0.025 nmol/L, 20 times lower than the IA. Analytical performance was within generally acceptable limits for routine clinical LC-MS/MS assays. In prostate cancer patients, for testosterone concentrations <1 nmol/L, differences ranged from -110.9% to 89.79% between the IA and LC-MSMS assay. Furthermore, in <1% vs 41% of urologist requests and 41% vs 91% of oncologist request, testosterone was below the LLOQ of the LC-MSMS assay and IA respectively. **Conclusion:** The LC-MS/MS based steroid panel was validated to properly assess in-house clinical indications. Notably, the sensitive testosterone analysis was able to quantify testosterone concentrations in a significantly larger set of prostate cancer patient samples than commercial IAs. Sensitive LC-MS/MS based testosterone analysis seems to be preferred for (chemically) castrated prostate cancer patients, though future studies are required to investigate its clinical utility.

A-366

Development of a Biochip Array for the Simultaneous Detection of Cancer Biomarkers on the Random Access, Fully Automated Evidence Evolution Analyser

P. Dunne[1], K. Martin[1], C. O Leime[1], C. Richardson[1], R. I. McConnell[2], J. V. Lamont[2], S. P. FitzGerald[2]. [1]*Randox Teoranta, Dungloe, Ireland,* [2]*Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: The serial measurement of circulating tumour biomarkers has found applications in screening of asymptomatic individuals and in efficacy monitoring of systemic cancer therapy as an inexpensive alternative to imaging modalities. However, the heterogeneity of tumour types and biomarker expression profiles has led to the necessity for analysis of multiple biomarkers to generate a comprehensive and accurate profile of an individual patient. Among the most commonly used cancer biomarkers are Cancer Antigen (CA) CA15-3, which has utility in the management of metastatic breast cancer; CA 125 is used for monitoring therapy in patients with ovarian cancer, carcinoembryonic antigen (CEA), used in surveillance of patients with diagnosed colorectal cancer; and both alpha-fetoprotein (AFP) and total beta- human chorionic gonadotrophin (hCG) are used in the management of patients with germ cell tumours including liver and testicular cancer. Biochip array technology allows the simultaneous detection of multiple analytes from a single sample, thus increasing the test result output per sample. The objective of this study was to develop a biochip array for the simultaneous measurement of CA15-3, CA125, AFP, CEA, and total beta hCG from a single sample for application to clinical settings. The use of the fully automated, random access biochip analyser Evidence Evolution enables high-throughput and rapid analysis.

Methods: Simultaneous chemiluminescent sandwich immunoassays were employed and applied to the Evidence Evolution analyser. Assay ranges were determined, and WHO Reference materials were employed for assay standardisation for AFP, CEA and total beta-hCG. Assays for CA15-3 and CA125 were standardised against reference methods on a commercially available platform. Sensitivity was determined under Clinical and Laboratory Standards Institute (CLSI) guidelines. A comparison study between the developed biochip array and reference methods or commercially available methodologies was completed by sample assessment.

Results: The analytical evaluation showed the following sensitivity values: 1 IU/mL (CA15-3, assay range: 0-250 IU/mL); 0.7 IU/mL (CA125, assay range: 0-800 IU/mL); 0.26ng/mL (AFP, assay range: 0-250 ng/mL); 0.3ng/mL (CEA, assay range: 0-250 ng/mL) and 1 mIU/ml (total beta-hCG, assay range: 0-2500 mIU/mL). The method comparison showed the following correlation coefficients: R^2: 0.8487 (CA15-3, n=33), R^2: 0.977 (CA125, n=33), R^2: 0.9493 (AFP, n=33), R^2: 0.8864 (CEA, n=45), and R^2: 0.9134 (total beta-hCG, n=33).

Conclusion: The data indicates optimal analytical performance of the biochip based simultaneous immunoassays for the measurement of five cancer biomarkers, when applied to the Evidence Evolution analyser. This multi-analytical application facilitates the generation of a comprehensive and accurate profile of an individual patient.

A-367

Development of a NSE Assay for Use on the ARCHITECT for Quantitation of Human Neuron Specific Enolase

A. Vasko[1], L. Zhu[1], B. Williams[1], C. Glover[1], E. Cawley[1], A. Motchenbacher[1], J. Ramirez[1], J. Hodgson[1], S. Khokhar[1], M. Barrett[1], A. Bartilomo[1], T. O'Kane[1], R. Frescatore[1], B. Lorelli[1], C. Traynham[1], C. Fermer[2], R. Smalley[1], G. Thorne[1]. [1]*Fujirebio Diagnostics Inc, Malvern, PA,* [2]*Fujirebio Diagnostics AB, Gothenburg, Sweden*

Introduction: Neuron Specific Enolase (NSE) is a glycolytic enzyme (2-phospho-D-glycerate hydrolase). It is an isoform of the enzyme enolase that is found in neurons and cells of endocrine origin. It may also be found in non-neuroendocrine cells, including but not limited to bronchial epithelial, pancreatic epithelial, secretory cells of the fallopian tube, plasma cells, lymphocytes, smooth muscle cells of the uterus, and various blood vessels. The NSE assay is to be used for the determination of NSE in the monitoring of response to therapy and detection of recurrent disease, such as small cell lung carcinoma (SCLC) and neuroendocrine tumors. **Methods:** The NSE assay for use on the ARCHITECT (in development) is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative measurement of NSE in human serum on the ARCHITECT *i* System. This method utilizes paramagnetic microparticles coated with a highly specific monoclonal antibody (mAb), which captures NSE present in the specimen. The microparticle, the sample and acridinium-labeled anti-NSE mAb conjugate are pipetted together into the reaction vessel. After an incubation and wash cycle, pre-trigger and trigger solutions are added and the resulting chemiluminescent

reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of NSE present in the specimen and the RLUs detected by the ARCHITECT *i* System optics. **Results:** Across three lots, the NSE assay demonstrated linearity from 1.6-400.0 ng/mL. The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the NSE assay were 0.1 ng/mL, 0.3 ng/mL and 0.6 ng/mL, respectively. A twenty-day precision study using 3 controls and 5 panels assayed twice per day (n=80 for each sample) demonstrated within laboratory (total) imprecision of ≤ 2.6% for controls and ≤ 5.1% for panels. Method comparison of the NSE assay with a predicate device was performed using Passing-Bablok regression and resulted in a slope of 0.97, a y-intercept of -1.54 ng/mL, and 0.990 correlation coefficient (r) across the assay range of 2.6 - 313.7 ng/mL. A tube-type equivalence study was performed between serum separator II tubes and primary serum red-top tubes A slope of 1.029 was observed. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 7 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum album, human anti-mouse antibody, and rheumatoid factor) and 20 commonly used therapeutic drugs (Paclitaxel, Docetaxel, Gemcitabine, Vinorelbine, Irinotecan, Vinblastine, Erlotinib, Pemetrexed, Cisplatin, Carboplatin, Doxorubicin, Vincristine, Ifosfamide, Cyclophosphamide, Teniposide, Epirubicin, Methotrexate, Busulfan, Topotecan, Biotin). Cross-reactivity of the NSE assay with αα NSE, non-neuronal enolase (NNE) targeted to 900 ng/mL showed cross-reactivity from 0.0-0.2%. There was no high-dose hook effect observed for samples containing up to ~160,000 ng/mL of NSE. The NSE reagents demonstrated on board stability and calibration stability on the instrument for 30 days. **Conclusions:** These data demonstrate that the NSE assay for use on ARCHITECT is sensitive, accurate and precise for the quantitative determination of NSE in serum specimens.

A-368

Development of Alternating Current Electrokinetics (ACE) Assays for the Quantification of Cell-Free DNA

R. Kovelman[1], P. F. Desmond[1], J. Hinestrosa[1], I. V. Dobrovolskaia[1], D. Ye[1], M. M. Nguyen[1], A. D. Kinana[1], R. P. Turner[1], D. Bodkin[2], R. Krishnan[1]. [1]*Biological Dynamics, San Diego, CA,* [2]*Cancer Center Oncology Medical Group, La Mesa, CA*

Current workflows to isolate complex analytes such as circulating nucleic acids in biological fluids are labor-intensive and can introduce variability into the analysis. Thus, there is a need for improved methodologies to simplify isolation and quantification, and toward this goal we have developed a novel technology based upon Alternating Current Electrokinetics (ACE) for the quantification of high molecular weight cell-free DNA (hmw cfDNA).

The core of our ACE system is a purpose-built microelectrode array embedded in a single-use device which, when inserted into a dedicated instrument providing the appropriate mechanical, microfluidic, electrical, thermal, and optical controls, enables the visualization of fluorescently labeled hmw cfDNA. In order to convert the fluorescence images into intensity measurements reflecting the concentration of hmw cfDNA, we developed a linked set of image processing and analysis algorithms. This set of algorithms is based on Canny edge detection and spatio-temporal sampling; it extracts the fluorescent signal arising from the labeled DNA and performs the dynamic background correction needed to work directly on opaque biological fluids.

We used our ACE system and image processing algorithms to determine hmw cfDNA concentrations directly in plasma processed from whole blood without additional sample purification or processing. Starting with purified DNA exogenously added to matrices with defined chemical compositions and then moving on to plasma, with or without exogenous DNA, we performed experiments to determine analytical parameters in the first implementation of this novel assay system. We observed linearity as a function of DNA concentration up to at least 250 pg/µL. At higher concentrations, the divergence from linearity was seen to be alleviated by the inclusion of higher concentrations of the fluorescent detection reagent. Combined with preliminary experiments to determine the limit of detection, for which we obtained values in the range of 4-10 pg/µL, this assay is capable of directly determining hmw cfDNA concentrations over a range of at least two orders of magnitude. This range covers the vast majority (at least 80-90%) of concentrations of hmw cfDNA found in plasma samples from the healthy normal individuals and cancer patients that we have assessed.

We have conducted studies to apply our ACE assays toward gaining a better understanding of the clinical relevance of levels of hmw cfDNA to human health and disease conditions. Studies involving Non-Small Cell Lung Cancer (NSCLC) patients, including those on immunotherapies, are underway. Interim analyses are indicative of clinically meaningful correlations between changes in levels of hmw cfDNA and patient condition. We will present updated results on our clinical findings and their relevance for the future use of ACE assays for these and other disease areas.

A-369

Neuron Specific Enolase Serum Collection, Processing Conditions, and Storage

A. Vasko, B. Williams, L. Zhu, A. Motchenbacher, J. Hodgson, D. Cline, G. Thorne. *Fujirebio Diagnostics Inc, Malvern, PA*

Introduction: Neuron Specific Enolase (NSE) may be used to support diagnosis or monitor clinical progression of neuroendocrine origin tumors such as retinoblastoma, neuroblastoma and small cell lung cancer (SCLC). NSE is also present in erythrocytes, plasmacytes, and platelets. Therefore, hemolysis could introduce an artificial elevation of NSE in patient samples. To limit hemolysis effects, the NACB Guidelines[1] recommend collection of serum within 60 minutes of venipuncture. This guidance, however, conflicts with serum tube manufactures' instruction for minimal clotting time (e.g. the time between venipuncture and centrifugation) of 60 minutes. The goal of the present study was to evaluate the effects of extended clotting times, as well as on-clot storage (time between centrifugation and serum collection), on NSE recovery. Furthermore, specimen storage conditions are recommended as 2-8°C and -70°C. A second aim was to evaluate storage times at room temperature and -20°C, since most clinical laboratories do not have access to -70°C freezers. **Methods:** Whole blood, freshly drawn from volunteers into red top serum collection tubes was allowed to clot for 60, 90, 120, 180, and 240 minutes. The serum was collected immediately from the clot, and assayed using an automated NSE assay in development. Differences in NSE concentration from the 60-minute clotting (NACB recommendation) were evaluated. To assess on-clot serum storage, whole blood was clotted for 60 minutes, centrifuged, and the serum samples were also allowed to sit in contact with the clot for 0, 1, 2, 4, or 6 hours at room temperature post-centrifugation. Differences in NSE concentration from the 0-minute contact were evaluated. Off-clot serum samples were stored for various times at room temperature and 2-8°C, and freeze/thaw cycled at -20°C or -70°C. Assayed results were compared to serum that was analyzed immediately after processing. Differences in NSE concentration from the fresh, nominally collected serum were evaluated. **Results:** The study results demonstrate that specimens centrifuged up to 90 minutes beyond venipuncture differed from their respective controls by <10%. Specimens stored on-clot up to 4 hours beyond centrifugation also showed <10% difference compared to nominal. Specimens stored at room temperature for up to 24 hours differed <10% from fresh serum. Specimens stored refrigerated (2-8°C) up to 48 hours also differed <10% from control serum. Specimens stored frozen at -70°C differed <10% from control serum, however specimens stored at -20°C differed by >10%. **Conclusions:** NSE specimens require stringent processing during collection to minimize known effects of hemolysis on endogenous NSE concentrations. The NACB guideline provides a conservative estimate for collection, processing, and storage of samples. We confirmed that 2-8°C and -70°C storage are the preferred storage conditions for NSE, and demonstrated that an extended clotting time up to 90 minutes is acceptable. In addition, we demonstrated that sera stored up to 4 hours in direct contact with the clot at room temperature are acceptable for use. 1 Petra Stieber, et al. National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Lung Cancer. Tumor Biology, 2006.

A-370

A Novel Integrated Digital PCR Platform for Precision Monitoring of BCR-ABL1 Transcripts

M. Dueck, R. Lin, A. Zayac, S. Gallagher, J. Kenemuth, P. Hung. *COMBiNATi, Burlingame, CA*

This study highlights the utility of a novel integrated digital PCR (dPCR) platform for highly precise, absolute quantification of BCR-ABL1 transcripts with a Deep Molecular Response of MR4.0 enabling a new paradigm for cancer recurrence monitoring.

95% of Chronic Myeloid Leukemia (CML) cases are characterized by the presence of a BCR-ABL1 fusion gene. Wildtype ABL1 is a tyrosine kinase that plays a role in cell cycle regulation and cellular differentiation. Expression of the BCR-ABL1 fusion oncogene generates a constitutively active tyrosine kinase leading to uncontrolled cellular proliferation and eventually cancer. The advent of tyrosine kinase inhibitor (TKI) therapy has had a positive effect on CML patient outcomes. Regular monitoring involving precise quantification of BCR-ABL1 transcripts is integral to TKI treatment. Additionally, BCR-ABL1 transcript monitoring is a valuable tool for minimum residual disease management. The current gold standard for clinical BCR-ABL1 transcript quantification is Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) which has limitations. Digital PCR (dPCR) offers several advantages over qPCR including: higher precision, absolute quantification (without the need for a standard curve) and higher sensitivity (less prone to inhibition). Despite this, dPCR has yet to become the gold standard for clinical BCR-ABL1 transcript quantification. In

this study, we benchmark a novel one-step, fully integrated dPCR platform designed to facilitate the transition of dPCR from the research lab setting to the clinic. This platform consists of a novel micro-injection molded microchamber array for reagent partitioning and a single instrument that integrates reagent partitioning, thermal cycling and data acquisition. The platform was designed to have a qPCR-equivalent workflow and go from sample to results in less than sixty minutes. Furthermore, refinements in the array design, combined with novel fluidic manipulation enables near-zero “dead volume”, thereby minimizing wasted clinical sample. The instrument is dry (does not contact fluids) and contamination-free, making it an attractive instrument for clinical applications. Using commercially available BCR-ABL1 PCR chemistry, we highlight this dPCR platform’s utility for precision BCR-ABL1 monitoring. To determine the dynamic range of the dPCR platform we generated a curve for both WT ABL1 and mutant BCR-ABL1 using a dilution series of synthetic genetic standards. We showcase that we can precisely quantify BCR-ABL1 transcripts down to a 0.01% mutant-to-wild-type ratio creating a new paradigm for cancer recurrence monitoring. In this study we address some key barriers to clinical adoption of digital PCR and hope to pave the way for improved, dPCR-based BCR-ABL1 diagnostics.

A-371

Two Serum Lectin Reactive Biomarkers, AFP L3 M2BPGi, for Diagnosis of Hepatocellular Carcinoma

S. Lee, S. Jeong, E. Jeong, J. Lee, Y. Hong, K. Yoon, M. Cho. *Pusan National Univ. Yangsan Hospital, Yangsan, Korea, Republic of*

Backgroud: To overcome the limitation of conventional in-vitro hepatocellular carcinoma (HCC) diagnosis, we aimed to evaluate the usefulness of addition of two glycoproteins, lectin reactive AFP (AFP L3) and M2BPGi (Mac-2-binding protein glycosylation isomer), as complement for two conventional markers, AFP and PIVKA-II.

Methods: Serum specimens from 143 subjects were submitted to measurement of tumor markers to diagnose HCC in a tertiary hospital in Korea. Among them, 84 were HCC patients, 53 were patients with non-HCC liver disease including liver cirrhosis, chronic hepatitis, HBV or HCV carrier. The measurement of each biomarkers - total AFP, AFP-L3, PIVKA-II, M2BPGi were performed with DxI800 (Beckman Coulter, Inc), μTAS (Wako Life Sciences, Inc.), Architect i2000 (Abbott diagnostics) and HISCL-5000 (Sysmex Corporation) in respectively. A separate reference evaluation for M2BPGi was performed using residual serum specimens (n =49) of healthy individuals. Cutoff values of two conventional tumor markers were 9 ng/mL for AFP 40 mAU/mL for PIVKA-II by instructions of manufacturers. New cutoff values were used for the additional markers, 10% for the AFP-L3 and 1 cutoff index (COI) for M2BPGi.

Results: Diagnostic performance of calculated AFP-L3 concentration by μTAS was 66.0% sensitivity and 88.7% specificity. Combining AFP-L3 to conventional markers had a higher area under the receiver operating curve (AUROC), 0.822, than single markers, AFP 0.816 and PIVKA-II 0.620. Although the higher M2BPGi values showed in patients than reference value 0.78 as the 97.5th percentile, the addition of M2BPGi showed only small increase of AUROC to 0.85.

CONCLUSIONS: Although the methods and instruments are different, the addition of glycoprotein biomarker increased the performance than conventional two tumor markers for HCC. AFP-L3 and M2BPGi were good complementary glycoprotein marker for the diagnosis of HCC.

A-372

BST-2 Suppresses the Growth and Metastasis of Hepatocellular Carcinoma by Inhibiting MyD88/PI3K Signaling

Y. Zhao, D. LI, S. Feng, Z. Wang, C. Tao. *West China Hospital of Sichuan University, Chengdu, China*

Background: This study is to measure BST-2 expression profiles in hepatocellular carcinoma (HCC) tissues, and investigate its biological function *in vivo and vitro*, clarifing the molecular mechanism. Finally, this study is aimed to find a potential target for HCC molecular dignostic and therapy.

Methods: BST-2 expression were measured by Real-time PCR and western blot in 30 fresh HCC tissues adjacent nontumorous liver tissues (ANLT), respectively, and its location and expression were investigated by immunohistochemistry (IHC) in 96 cases of HCC. Employing Kaplan-Meier survival curves to calculate the overall survival rate(OS). The relationship between BST-2 expression and prognosis of HCC was evaluated by univariable and multivariable Cox proportional hazard regression model. Selecting suitable hepatoma cell lines to construct overexpression cells and knock down cells to investigate the role of BST-2 on proliferation, invasion and metastasis of HCC using MTT, wound healing assay and transwell assay in vitro, and to study the impact of BST-2 on the growth and invasion and metastasis of HCC by comparing the tumor size, intrahepatic metastasis and lung metastasis in vivo, demonstrating the biological function of BST-2 in HCC.

Results: BST-2 expression in 30 cases of HCC and ANLT by Real-time PCR showed that the median of BST-2 mRNAs in HCC was 0.314(0.227-0.603); BST-2 protein expression measured by western blot in HCC(0.263±0.454) was lower than those in their ANLT(0.772±0.460) (P<0.001), in parallel with BST-2 mRNA expression, located on the membrane and cytoplasm by IHC. Of 96 cases of HCC and its corresponding ANLT, the positive expression rates of BST-2 were 29.17%(28/96)and 79.17%(69/96). The difference was statistically significant (P<0.001). The BST-2 expression was related to tumor size (P=0.001), vein invasion (P=0021), capsule formation (P=0.001), and BCLC staging (P=0.009) by correlation analysis. The OS rates of patients with low and high expression of BST-2 were 0.0% and 0.11%, respectively. Tumor size(HR=4.530,P=0.001), vein invasion(HR=2.254,P=0.003), BCLC staging(HR=4.20,P=0.007)and low expression of BST-2(HR=4.077,P=0.026) were recognized as independent predictors of postoperative OS. The growth rates of Huh7^{BST-2} cells with BST-2 overexpression had more decreased than Huh7^{NC}(P<0.05), and the migration of Huh7^{BST-2} cells were slower than Huh7^{NC} cells (P<0.01). While the migration and invasion abilities of HepG2$^{siBST-2}$ cells with BST-2 knockdown were more enhanced than HepG2^{NC} cells (P<0.05).The subcutaneous xenograft tumor models showed that the nude mice in Huh7^{BST-2} group had smaller tumor size than those in Huh7^{NC} group in liver in situ with lower rates of intrahepatic and pulmonary metastasis (P=0.017).

Conclusion: This study firstly identified that BST-2 was down-regulated in HCC and its expression was closely correlated to tumor size, vein invasion, capsule information and BCLC staging. Furthermore, the low expression of BST-2 is an independent predictor of postoperative bad prognosis. Finally, this study demonstrated that BST-2 can suppress HCC cell proliferation and migration though regulating MyD88/PI3K signal pathway.

A-373

A Putative Gain-of-Function *JAK2* Germline Mutation Detected by NGS in a MPN *BCR-ABL1*-Negative Neoplasia

M. G. D’Andrea[1], W. D. Correia[1], F. Milanezi[2], S. Argolo[1], G. A. Campana[2], I. R. Zalcberg[1]. [1]*GeneOne, Dasa Brazil, Rio de Janeiro, Brazil,* [2]*GeneOne, Dasa Brazil, São Paulo, Brazil*

Background: Myeloproliferative Neoplasms (MPN) are clonal diseases leading to increased blood cell production. The 2016 WHO Classification categorized MPN into BCR-ABL1 positive - Chronic Myeloid Leukemia (CML) - and BCR-ABL1 negative - Chronic neutrophilic leukemia (CNL), Chronic eosinophilic leukemia (CEL), Classical MPN (Polycythemia Vera (PV), Essential Thrombocythemia (ET), Primary Myelofibrosis (PMF)), rare MPN and Mastocytosis. Signaling mutations in the JAK2/STAT pathway are major criteria for the diagnosis of PV, PMF and ET, and most often involve genetic evaluation of JAK2 in exon 14 (especially V617F), or in exon 12 in PV, CALR and MPL providing criteria for the diagnosis of MPN. Detection of driver mutations excludes a reactive process. Most cases of CNL and PV either have CSF3R (>80%) or JAK2 (~98%) mutations. Several other acquired JAK2 mutations have now been shown to contribute to the pathogenesis of MPNs, among them, rare germline mutations. Next Generation Sequencing (NGS) allows detection of mutations and copy number variation in 30 genes involved in the development of myeloid neoplasms.

Objective: We aimed to sequence a panel of 30 genes associated with Myeloid Neoplasms to aid in the diagnosis of a MNP BCR-ABL1 negative sample and to exclude a reactive process. The reported patient was referred in 2018 for molecular screening to the genetic branch of a private laboratory, because of MPN suspicion. This study was carried out in accordance with the World Medical Association Declaration of Helsinki.

Methods: Genomic DNA was extracted from a peripheral blood sample. DNA libraries were prepared with Myeloid Solution, by Sophia Genetics. DNA was submitted to enzymatic fragmentation, adapter ligation, size selection, targeted hybridization and amplification. Sequencing was performed on MiniSeq (Illumina) with 150bp paired-end reads. Data were analyzed using Sophia DDM software with a specific algorithm for variant calling and annotation. Target regions were covered at least 1000X. Thirty genes were analyzed: ABL1, ASXL1, BRAF, CALR, CBL, CEBPA, CSF3R, DNMT3A, ETV6, EZH2, FLT3, HRAS, IDH1, IDH2, JAK2, KIT, KRAS, MPL, NPM1, NRAS, PTPN11, RUNX1, SETBP1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1, ZRSR2.

Results: The patient presented a possible germline gain-of-function mutation in JAK2 exon 9 (c.1177C>G, p.L393V), but no somatic driver mutations in JAK2, CALR or

MPL were found as commonly described in MNP BCR-ABL1 negative patients. Additional mutations were detected in CSF3R (c.958G>A, p.D320N)

and SETBP1 (c.691G>C, p.V231L) and may have also contributed to the MPN pathogenesis.

Conclusions: NGS detected a putative gain-of-function JAK2 germline mutation without involvement of JAK2 V617F in an MPN BCR-ABL1-negative neoplasia. MPN-like hereditary diseases may be caused by germline JAK2 mutations and, in addition to the somatic JAK2 V617F mutation, they can also induce MPN phenotype (e.g., R564Q and V625F) or isolated thrombocytosis (V617I) and erythrocytosis (E846D and R1063H). Based on this analysis we speculate that germline mutations outside the core regulatory domain can also give rise to MPN-like hereditary diseases. However, the molecular basis of this process remains to be elucidated.

A-374

Evaluation of markB® Point-of-Care Immunoassay System for Determination of Tumor Markers: Prostate-Specific Antigen, Alpha-Fetoprotein, and Carcinoembryonic Antigen

H. Hwang[1], J. Kim[1], H. Kim[1], Y. Son[1], H. Shin[1], H. Han[2], J. Choi[1]. *[1]BBB Inc., Seoul, Korea, Republic of, [2]Seoul National University Bundang Hospital, Seongnam, Korea, Republic of*

Background: Tumor markers are substances in the body whose levels may be elevated by the presence of cancer. Prostate-specific antigen (PSA), alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA) are well known as serological tumor markers for prostate cancer, hepatocarcinoma, and colorectal cancer, respectively. Interests in point-of-care testing (POCT) of the tumor markers have been steadily grown due to the potential for POCT to improve healthcare quality through rapid, accurate, and on-site detection of disease markers using a tiny drop of body fluid. Recently, we have developed a novel immunoasssay platform, called markB®, which is based on passive plasma separation and magnetic force-assisted electrochemical sandwich immunoassay. Herein, analytical performances of markB® for the quantification of PSA, AFP, and CEA are evaluated.

Methods: Total 242 patient samples were collected and analyzed. Key performance testing including precision, limit of quantification (LoQ), linearity, interference, and method comparison with Roche cobas e 801 were accessed per the CLSI guidelines. For analysis, a strip is placed on a markB® reader, and 30 μL of specimen is loaded on the strip. Then whole assay processes, from sample preparation to electrochemical detection, are automatically conducted within 15 min.

Results: The results for precision, LoQ, method comparison, and defined measuring intervals for the assays of PSA, AFP, and CEA are summarized in the table below. The measurement value variations by more than 50 potential interferents, including bilirubin, hemoglobin, triglyceride, total protein, rheumatoid factor, and HAMA, were less than 10% at the clinically high concentration.

Conclusion: The immunoassays of representative tumor markers such as PSA, AFP, and CEA utilizing markB® showed acceptable analytical performances in the defined measuring ranges. The method comparison studies showed excellent correlation between markB® and Roche cobas e 801 for the three tumor markers. These results imply that markB® can be potentially used in clinical practices for the detection of tumor markers.

	Within-run Precision (%CV)	Total Precision (%CV)	LoQ (ng/mL)	Method Comparison to Roche cobas e 801 (Slope/r)	Measuring Interval (ng/mL)
PSA	1.33-6.56	3.94-9.75	0.1	1.037/0.997	0.1-30
AFP	1.13-4.01	3.19-8.64	1	0.966/0.996	1-700
CEA	1.55-4.96	3.77-7.71	1	1.042/0.996	1-500

A-375

A Novel Thyroglobulin Immunoassay using iTACT Method Improves Accuracy of Thyroglobulin Measurements in Anti-thyroglobulin Positive Specimens

Y. Kitamura[1], S. Narita[1], Y. Kuroda[1], S. Yagi[2], K. Aoyagi[3]. *[1]Fujirebio inc., hachioji, Japan, [2]Advanced Life-science institute INC., hachioji, Japan, [3]Fujirebio inc., Advanced Life-science institute INC., hachioji, Japan*

Background:

Interference by endogenous autoantibodies to thyroglobulin (TgAb), causing false-negative results, is a major issue in almost current thyroglobulin (Tg) immunoassays, therefore guidelines recommends TgAb measurements to assess validity of serum Tg values. LC-MS/MS for measuring Tg have been proposed alternatives for Tg immunoassay to monitor TgAb positive thyroid cancer patients. Although LC-MS/MS protocols have been shown to be resistant to the TgAb interference, they require longer turn around time (TAT) and more operation costs than conventional automated immunoassay systems.

iTACT (Immunoassay for Total Antigen including Complex via pre-Treatment) is a novel immune assay system consisting of a sample pretreatment step and a robust immunoassay, and have been applied to virus antigen measurements. After extraction of antigens by the pretreatment to disrupt antigen complex such as virus particles, capsids, the antigen complex with antibody and/or binding proteins, the pretreated antigen can be detected by the immunoassay accurately and precisely without interference.

In this study, we applied iTACT protocol to develop a novel Tg immunoassay on the fully automated chemiluminescent enzyme immunoassay (CLEIA) system (LUMIPULSE® L2400 system), and examined the performance of the established prototype assay by comparing those by other Tg measurement systems.

Methods:

All evaluations were executed with LUMIPULSE® L2400 analyzer installed with on-board pretreatment process (FUJIREBIO INC.). The prototype assay requires total 32.5 min running time including 6.5 min pretreatment process for an assay. The measurement values in calibrators for LUMIPULSE® L2400 analyzer were traceable to reference materials (BCR-457) for Tg. The sample types used in this study included both TgAb positive and negative serum and Li-heparin plasma. Within-run precision, limit of quantification (LoQ), dilution linearity and spike recovery, matched pair correlation between serum and plasma, correlation test with Elecsys Tg-II (Roche, Cobas) were evaluated based on recommendation in CLSI documents. Size exclusion chromatography (Superose 6 Increase 10/300 GL, GE) was executed by using one of TgAb positive specimens to reveal Tg free form by disrupting TgAb/Tg immune-complex.

Results:

Within-run precision CVs for our assay ranged from 1 to 3% when 3 different conc. of quality controls were tested, LoQ was calculated at 0.03 ng/mL by precision profile. Dilution linearity and spike recovery showed excellent performance. Correlation between serum and heparin Li-plasma with 60 matched pair samples was excellent (slope: 0.953, regression: 0.997). The significant

correlation with Elecsys Tg-II using 54 specimens of TgAb negative was observed (slope: 1.007, regression: 0.999). TgAb positive specimens (range of TgAb titer: 30-3,000 IU/mL, n=177), in contrast, showed many discrepant data on bias-plot to Elecsys Tg-II values. The effects of pretreatment of samples were evaluated with size exclusion chromatography. Immuno-reactive materials in a pretreated discrepant specimen were detected fractions corresponding to the molecular mass of free Tg (660 kDa) in contrast to those corresponding to higher molecular mass from the non-treated specimens, indicating extraction of free Tg from TgAb-Tg immune-complexes by pretreatment.

Conclusion:

Our study indicated that iTACT Tg immunoassay prototype would be robust to interferences by TgAb, and would be able to provide an accurate Tg concentration easily instead of LC-MS/MS.

A-376

Evaluation of Hyaluronic Acid Assay on Abbott Architect Chemistry Analyzer in Patients with Cirrhosis and Hepatocellular Carcinoma

W. Tamimi[1], I. Altraif[2], T. Bautista[1], J. Rodriguez[1], A. Alsaghir[1], A. Aljumah[2], N. Alotabi[1]. *[1]Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, King Saud Bin Abdulaziz University for Health Sciences, King Abdullh International Research Center, National Guard, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia, [2]Department Hepatobiliary Sciences, King Abdulaziz Medical City, King Saud Bin Abdulaziz University for Health Sciences, King Abdullh International Research Center, National Guard, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia*

Background: Hyaluronic acid (HA) is an unbranched glycosaminoglycan, a single chain of polymers of disaccharide units which is widely distributed in connective tissue and is produced mainly in mesenchymal cells. HA has been used to assess liver cirrhosis. It is also consistent with the stage of fibrosis and thus, may be useful for non-invasive hepatic fibrosis assessment. However, this method is a manual assay and requires time and more effort to be performed. Therefore, the aim of this study was to

evaluate and validate this method on an open channel of an automated general clinical chemistry analyzer which can produce more accurate and faster results. **Methods**: This was a prospective study in which blood samples were collected from 73 patients from liver disease clinics. All patients were above age of 18 years. All patients were clinically assessed and diagnosis were confirmed either clinically or by radiological as well as liver biopsy and fibroscan. Patients were divided into four groups as first group chronic hepatitis without liver cirrhosis (25 patients) in which 18 were with hepatitis B.; second group chronic hepatitis patients with cirrhosis (37 patients) in which 32 were with hepatitis B; third group were patients with hepatocellular carcinoma (HCC) (11 patients), and the fourth were healthy donors control group (11 patients). The HA assay (FUJIFILM Wako Chemicals, Germany) is based on the latex agglutination turbidimetric method and has been applied to general clinical chemistry analyzer Architect C16000 (Abbott, USA). The alphafetoprotein (AFP) was measured by chemiluminescent microparticale immunoassay (CMIA) Architect i2000 (Abbott, USA). The reference range for HA was 6.0 - 40.0 ng/mL and for AFP was ≤ 8 ng/mL. **Results**: The first group have showed that 11/25 (44%) patients had abnormal HA with diagnostic sensitivity and specificity of 44% and 100% respectively. The second group had abnormal HA results 31/37 (84%) with sensitivity and specificity of 84% and 100% respectively. The third group had abnormal results in 9/11(82%) patients with sensitivity and specificity of 82% and 100% respectively. The AFP has shown lower sensitivity of 0% , 14%, and 18% but higher specificity of 100% for three groups respectively. **Conclusion:** The HA assay was applied on an open channel of the Architect chemistry analyzer from Abbott and has successfully reduced the turnaround time for reporting HA. The diagnostic utility of HA has shown to be superior to AFP in evaluating patients with cirrhosis and with HCC. Due to some limitations of this study, further studies are needed to establish solid conclusion for the clinical utility of HA.

A-377

Comparison of Two Methods for Determination of Hyaluronic Acids in Blood for Patients with Liver Diseases

W. Tamimi[1], I. Altraif[2], T. Bautista[1], J. Rodriguez[1], A. Alsaghir[1], A. Aljumah[2], N. Alotabi[1]. *[1]Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, King Saud Bin Abdulaziz University for Health Sciences, King Abdullh International Research Center, National Guard, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia, [2]Department Hepatobiliary Sciences, King Abdulaziz Medical City, King Saud Bin Abdulaziz University for Health Sciences, King Abdullh International Research Center, National Guard, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia*

Background: Hyaluronic acid (HA) is an unbranched glycosaminoglycan, a single chain of polymers of disaccharide units which is widely distributed in connective tissue and is produced mainly in mesenchymal cells. HA has several functions, including lubrication in joints, prevention from bacterial invasion and internal body hydration. HA has been used to assess liver cirrhosis. It is also consistent with the stage of fibrosis and thus, may be useful for non-invasive hepatic fibrosis assessment. However, results of various methods are discordant and HA assays are not standardized. Therefore, the aim of this study was to compare and clinically validate two available methods from two different manufacturers for HA. **Methods**: A total of 42 samples were used to evaluate of the two methods. Additional 11 samples from healthy donors were also used as a control. All patients were above age of 18 years. All patients were clinically assessed and diagnosis were confirmed either clinically or by radiological as well as liver biopsy and fibroscan. Patients were divided into four groups as first group chronic hepatitis without liver cirrhosis (17 patients); second group chronic hepatitis patients with cirrhosis (16 patients); third group were patients with hepatocellular carcinoma (HCC) (9 patients), and the fourth were healthy donors control group (11 patients). The HA assay (FUJIFILM Wako Chemicals, Germany) is based on the latex agglutination turbidimetric method and has been applied to general clinical chemistry analyzer Architect C16000 (Abbott, USA). The alphafetoprotein (AFP) was measured by chemiluminescent microparticale immunoassay (CMIA) Architect i2000 (Abbott, USA). The second method was competitive chemiluminescent immunoassay (CLIA) MAGLUMI (Snibe, China). The reference range for HA Architect and MAGLUMI were 6.0 - 40.0 ng/mL and <100 ng/mL respectively. The reference for AFP was ≤ 8 ng/mL. **Results**: There were a 93% total agreement with correlation of r=0.917 (p=0.0582) between the two assays based on its assay cutoff. However, there was a large bias of -77% (p<0.001) between the two assays, possibly due to different methodologies and different cutoff values. The clinical sensitivity for HA on Architect were found to be 53%, 75% and 89% for the first, second and third group respectively; compared to sensitivity on MAGLUMI 41%, 75, and 78% respectively. All have shown 100% specificity. **Conclusion**: The two HA methods Architect C16000 using FUJIFILM Wako and MAGLUMI immunoassay are comparable and agreed with each other in evaluating patients with cirrhosis and HCC. The best clinical utility of this test is to rule out any liver diseases rather than to rule in.

A-378

Evaluation of Blood Measurements of Des-gamma-Carboxy Prothrombin and Lens Culinaris agglutinin A-reactive Alpha-Fetoprotein in Patients with Susbected Hepatocellular Carcinoma

W. Tamimi[1], I. Altraif[2], A. Alsaghir[1], M. Alshaibani[1], S. Mahmoud[1], A. Aljumah[2], N. Alotabi[1]. *[1]Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, King Saud Bin Abdulaziz University for Health Sciences, King Abdullh International Research Center, National Guard, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia, [2]Department Hepatobiliary Sciences, King Abdulaziz Medical City, King Saud Bin Abdulaziz University for Health Sciences, King Abdullh International Research Center, National Guard, Riyadh, Saudi Arabia, Riyadh, Saudi Arabia*

Background: Patients with liver diseases are at risk of developing Hepatocellular carcinoma (HCC). Physicians normally follow up their patients by ultra sound image, liver biopsy and non-invasive biochemical markers such as alpha-fetoprotein (AFP). However, AFP is less sensitivity and not specific. New markers such des-gamma-carboxy prothrombin (H-DCP) and Lens culinaris agglutinin A-reactive alpha-fetoprotein (AFP-L3) have been developed and yet need more clinical validation. In this study we have tried to evaluate these two markers in our patients. **Methods**: Blood samples were collected from 28 patients at liver clinics. Samples were transported to the lab, separated and stored. All patients were clinically assessed and diagnosis were confirmed either clinically or by radiological as well as liver biopsy and fibroscan. DCP was measured by electrochemiluminescence immunoassay (cut-off 7.5 ng/mL)) and AFP-L3% (percentage of AFP-L3/total AFP) by lectin-affinity electrophoresis coupled with the antibody-affinity blotting method (cut-off 10%). Both were measured by µ TASWAKO 130 analyzer (Wako, Germany). The total alphafetoprotein (AFP) was measured by chemiluminescent microparticale immunoassay (CMIA) Architect i2000 (Abbott, USA) as well as by µ TASWAKO 130 analyzer. **Results**: Of 28 patients, 3 (10.7%) were positive for H-DCP and 4 (14.3%) were positive for AFP-L3. Two patients were confirmed to have HCC. There was no correlation between DCP and AFP-L3%. However, there were a good correlation (r=0.9968; p=0.1715) between µ TASWAKO 130 analyzer and Architect i2000 for total AFP. The diagnostic sensitivity and specificity for DCP were found to be 50% and 92% respectively and for AFP-L3 were found to be 100% and 92% respectively. **Conclusion**: The detection of HCC by DCP and AFP-L3% were evaluated in our patients. Our results indicate that these two markers are complementary and useful for the diagnosis and evaluation of HCC. However, more samples, time and further studies are needed to establish solid conclusion about the clinical utility of these two markers.

A-379

Prospective Comparison of CellDetect with Urine Cytology and Urovysion-FISH for Diagnosis of Bladder Cancer

K. Gueta Milshtein[1], B. Wilchfort[1], I. Masarwe[2], J. Bejar[3], H. Shefer[3], I. Bejar[2], S. Halachmi[2], K. Biton[2], O. Nativ[2]. *[1]Micromedic Technologies Ldt, Tel Aviv, Israel, [2]Department of Urology, Bnai Zion Medical Center, Haifa, Israel, [3]Department of Pathology, Bnai Zion Medical Center, Haifa, Israel*

Background: Accurate detection urothelial carcinoma may be challenging, particularly in low-grade cases where cytomorphologic features overlap with those of non-neoplastic changes. To increase diagnostic accuracy, standard urine cytology is often done in conjunction with biomarkers. CellDetect ®, a novel urine-based biomarker, is based on histochemical stain platform that uses a color feature to highlight neoplastic cells in urine specimens resulting in high performance throughout all cancer grades and stages. The objective of the present study was to confirm this finding in routine clinical settings and to compare standard urine cytology to CellDetect® and Urovysion-FISH performance. **Methods:** Patients undergoing routine cystoscopic surveillance or TURBT were enrolled in the study. Voided urine samples were preserved and processed into 3 cytocentrifuge smears: CellDetect®, standard cytology stain (PAP) and Urovysion- FISH. The smears were blindly reviewed by a cytopathologist. The performance of all three techniques was then compared to gold standard (biopsy for positive cases and cystoscopy for negative cases). **Results: :** The study included 86 patients, including 43 negatives and 43 positive cases confirmed by gold standard. The diagnostic accuracy of each test was computed based on determined readings and showed: CellDetect® correctly identified 89% of the positive cases while standard cytology and Urovysion-FISH identified only 47% and 62% of the cases, respectively. The specificity of CellDetect® was 83%, standard cytology 95% and Urovysion-FISH

78%. 12 cases were categorized as atypical by standard cytology; within these cases, CellDetect® identified 3 true positive cases and 3 true negative cases, resulting in 100% sensitivity and specificity. **Conclusion:** This study validates the use of Cell-Detect in routine clinical settings. CellDetect® outperforms both urine cytology and Urovysion-FISH sensitivities in detection of bladder cancer. Moreover, by reducing the number of atypical results, CellDetect® contributes to a more precise choice of treatment for the patients. Thus, testing patients with CellDetect® will aid in a confirmatory diagnosis of Bladder cancer.

A-380

Prostate-Specific Antigen (PSA) Test Use in the United States for Men Age >=65 Years, 1999-2015: Implications for Practice Interventions

K. P. Sharma, D. A. Siegel. *Centers for Disease Control and Prevention (CDC), Atlanta, GA*

Background: The U.S. Preventive Services Task Force (USPSTF) has issued several recommendations discussing use of the prostate-specific antigen (PSA) test to screen for prostate cancer. In 2003, USPSTF revised its 1996 recommendation against PSA screening noting that there was insufficient evidence to make any recommendations. In 2008, the USPSTF recommended against screening of men age ≥75, with no recommendation for younger men. In 2012, the USPSTF recommended against PSA screening of all men. The objective of this study was to estimate the rate of PSA test use in men age ≥65 from 1999 through 2015 and provide more recent data than past studies using similar claims-based methodology.

Methods: Medicare claims data were accessed for men age ≥65 years (9.7-11.1 million men) using the virtual research data center of the Centers for Medicare & Medicaid Services. Crude rates for PSA testing in each calendar year were the proportion of included men with ≥1 PSA test(s) per 12 months of continuous enrollment. PSA claims associated with ≥1 prostate-related conditions or non-laboratory procedures were excluded. We determined trends in PSA estimated screening rate by age group in 1999-2015 using joinpoint regression analysis. Annual percent change (APC) was estimated for various line segments by fitting annual data to identify the log-linear model with the fewest number of inflection points. Only significant trends - APC values associated with $P < 0.05$ - were listed along with P for trend.

Results: During 1999-2015, the range of estimated PSA test use expressed as minimum-maximum annual rates for each age grouping were: 10.1%-23.1% (age ≥85), 16.6%-31.0% (age 80-84), 23.8%-35.8% (age 75-79), 28.3%-36.9% (age 70-74), and 26.4%-33.6% (age 65-69). Significant APC trends were: $APC_{1999-2002}$ = 8.1% (P = 0.029) and $APC_{2008-2015}$ = -9.0% ($P < 0.001$) for men age ≥85, $APC_{2008-2015}$ = -7.1% ($P < 0.001$) for men age 80-84, $APC_{2001-2015}$ = -2.5% ($P < 0.001$) for men age 75-79, $APC_{2008-2015}$ = -3.3% (P = 0.007) for men age 70-74, and $APC_{2010-2015}$ = -5.2% (P = 0.014) for men age 65-69. For men age ≥70, the PSA test use rate decreased from 1999 to 2015 by 40.7%, 29.9%, 13.9%, and 2.9%, respectively, for men aged ≥85, 80-84, 75-79, and 70-74 ($P < 0.001$). For men age 65-69, the PSA estimated test use rate increased from 1999 to 2015 by 0.3% (P = 0.039).

Conclusion: Substantial proportions of men are receiving PSA test, especially those in the ≥75 age group (from 10%-23% in men age ≥85 to 28%-37% in men age 70-74). One of the reasons for the high rate of PSA test use, even after excluding the tests for which PSA test is indicated (e.g. history of prostate cancer), might be that PSA test is being used as screening for prostate cancer. Further research is needed to understand why PSA testing is performed, particularly in older men. Future research could also examine if the decreasing trend in PSA screening continues in alignment with current evidence-based recommendations.

A-381

Comparison of Electrophoretic Systems to Detect Occult IgA Monoclonal Immunoglobulins

P. MULUHNGWI[1], T. Yin[1], K. M. Cost[2], R. J. Elin[1]. [1]*University of Louisville, LOUISVILLE, KY,* [2]*Northon Healthcare, LOUISVILLE, KY*

Background: Monoclonal immunoglobulins (MI) are diagnostic and prognostic biomarkers for several clonal diseases including multiple myeloma. These antibodies (IgG, IgA, IgM, IgD, and IgE) are secreted by mature plasma cells. Approximately 3-4% of patients with multiple myeloma do not produce or secrete monoclonal proteins. MIs are screened using serum protein electrophoresis (SPE), where proteins are separated in an agarose gel based on their electrophoretic mobility and charge distribution of the protein to produce a pattern of major fractions: albumin, alpha-1, alpha-2, beta, and polyclonal gamma globulins. MIs are present in the gamma globulin region, but can also be found in the beta region or alpha-2, which can make quantitation and interpretation inaccurate. Elevated beta globulin, of which the major fraction is transferrin, may be an indication for iron deficiency anemia, 3rd trimester pregnancy, or the use of oral contraceptives. Capillary electrophoresis (CE) can resolve the beta-globulin as two peaks; $beta_1$ (transferrin) and $beta_2$ (complement proteins [C3]) regions. We present three cases that illustrate the resolving power of these two methods to detect the presence of an IgA MI band hidden within the beta region. **Method:** SPE and CE were performed using the Helena SPIFE 3000 and Sebia Capillarys2 systems respectively. Protein fractions were quantified by densitometry (Helena) with total serum protein concentration as the reference or direct UV absorbance (Sebia) at 200 nm. The presence of a MI was confirmed by immunofixation electrophoresis (IFE). The Beckman DxC and Vitros 5,1 FS were used to quantitate immunoglobulins. Reference ranges: $beta_1$ (0.30 - 0.60 g/dL), $beta_2$ (0.20 - 0.50 g/dL). **Results:** Patient A showed a normal electrophoretic pattern and protein concentration from the SPE, but an elevated $beta_2$ (0.87 g/dL) versus normal $beta_1$ (0.45 g/dL) using the CE method. Immunoglobulin quantitation methods (IQMs) showed an elevated IgA (Beckman: 677; Vitros: 634 mg/dL), and normal IgG and IgM - IFE confirmed an IgA lambda. Patient B showed an elevated beta globulin but otherwise a normal electrophoretic pattern from the SPE. The CE method showed an elevated $beta_2$ (1.0 g/dL) versus a normal $beta_1$ (0.42 g/dL). IQMs showed an elevated IgA (Beckman: 913; Vitros: 851 mg/dL), normal IgG and IgM - IFE confirmed an IgA lambda. Within the beta region, Patient C showed an elevated monoclonal protein adjacent to the beta globulin peak on the SPE. The CE method showed an elevated $beta_2$ (1.6 g/dL) versus a normal $beta_1$ (0.38 g/dL). IQMs showed an elevated IgA (Beckman: 1,177; Vitros: 1,122 mg/dL), normal IgG and IgM - IFE confirmed an IgA lambda. **Conclusion:** Our results demonstrate that the capillary method is superior to agarose gel electrophoresis due to its ability to separate the beta region into $beta_1$ and $beta_2$, thus allowing better detection of hidden IgA MIs. Furthermore, we observe a pattern whereby the concentration difference between $beta_1$ and $beta_2$ become reversed ("flipped") in the presence of a hidden IgA MI. Further studies with a larger sample size may be warranted to establish a rule for IFE reflexing when the $beta_2$ fraction is greater than the $beta_1$.

A-382

A Multi-Cancer Panel Screening from Solid Tumors in a Triennium Brazilian Cohort

N. P. Lopes[1], M. A. Pereira[2], R. L. M. Guedes[2], N. L. Viana[1], D. Zauli[2], M. C. M. Freire[1]. [1]*Hermes Pardini Institute (Progenética Laboratory), Belo Horizonte, Brazil,* [2]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Next-Generation Sequencing (NGS) technology is enabling the clinical diagnosis to benefit from personalized medicine, through concomitant analysis of several therapeutic-associated regions/genes of multiple patients in a single experiment. This is currently allowing a new era in modern medicine, leading to more precise cancer diagnostics, treatments and survival rates. **Objective:** To survey the spectrum of variants (hotspots, SNVs, indels, CNVs and gene fusions) found in 52 oncogenes from solid tumor samples using the multi-biomarker assay Oncomine Focus Assay (Thermo Fisher Scientific). **Methodology:** 649 Formalin-Fixed Paraffin-Embedded (FFPE) samples from diverse tumors (321 males and 328 females) and clinical-pathological data were obtained from pathologist/physician responsible for each patient. Total genomic DNA and RNA were extracted from all samples. Libraries were prepared and sequenced on Ion Torrent PGM and Ion S5 sequencers. Sequencing data was analyzed using Ion Reporter software integrated with Oncomine® Knowledgebase. **Results and Discussion:** Sampling was performed from the year 2016 to 2018. A total of 191 patients (29.4%) did not present any detectable variants whilst poor DNA/RNA quality and/or quantity caused 21 (3.2%) inconclusive results. Therefore, these patients were excluded from further analysis. In general, 427 point mutations/indels (68.6%), 124 CNVs (19.9%) and 71 gene-fusions (11.4%) were observed for the remaining 437 patients. Lung cancer was the most frequent tumor type observed (67.7%) followed by colorectal (6.6%) and breast (4.7%) cancers. For lung cancer, the most frequent variants were: i) SNV/indels: *KRAS* G12/G13 hotspot (30.6%), *EGFR* DEL19 (12.1%) and *EGFR* L858R (8.7%); ii) Gene-fusions: *EML4-ALK* (42.6%) and *MET* (13-15) (21.3%); iii) CNV: *CDK4* (23.4%) and *EGFR* (20.3%). For breast cancer, as expected *PIK3CA* was the most recurrently mutated gene (29.7%). *PIK3CA* point mutations were also frequent for lung cancer (10.19%) but it is still unclear whether they represent potential targets for EGFR-TKI therapy. Interestingly, this panel allowed the identification of not commonly observed variants in these tumors such as ovary adenocarcinoma *PIK3CA* p.M1043I, moderately differentiated colon adenocarcinoma *FGFR4* amplification, lung adenocarcinoma *BRAF* p.G469A and *ERBB2* amplification. All these alterations were previously associated with drug response for other cancer types. **Conclusions:** This study demonstrates the importance of a multi-cancer panels for clinicians and patients for the identification of multiple druggable variants with approved therapies or ongoing clinical trials, improving treat-

ment opportunities. Additionally, the screening of large tumor datasets contributes to the description of mutations classified as non-pathogenic that may eventually be reclassified as pathogenic driver mutations and also in the detection of new relevant variants. It is important to highlight that these observations should be interpreted with caution as treatment availability and physicians experience are a probable sampling bias.

A-383

Extracellular Vesicles Derived from HIV-Infected Patient Saliva Promote KSHV Infection and Transmission via the HIV TAR RNA

L. Chen[1], Z. Feng[1], G. Yuan[1], B. Reinthal[1], F. Ye[2], G. Jin[1]. *[1]Department of Biological Sciences, School of Dental Medicine, Case Western Reserve University, Cleveland, OH, [2]Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, OH*

Background: Kaposi sarcoma herpesvirus (KSHV) is a causal agent for Kaposi sarcoma (KS), a leading cause of mortality and morbidity in people living with HIV/AIDS. Despite advances in our understanding of virology and pathogenesis of KSHV, the predominant route of KSHV transmission and risk factors for infection are still not well understood. Extracellular vesicles (EVs) are lipid bilayer-enclosed nanoparticles released by many types of cells. HIV patient saliva contains a variety of immunologically active EVs to regulate the immune response of oral mucosal cells. We investigated the role of EVs derived from saliva of HIV-infected people under anti-retroviral therapy to identify HIV-specific mechanisms for the development of novel therapeutic approaches in people living with HIV (PLWH). **Methods:** Extracellular vesicles were isolated from HIV+ patient and healthy individual saliva using the ultracentrifugation protocol. EVs were validated by Transmission Electron Microscope (TEM) and western blotting for tetraspanins (CD9, CD63 and CD81). KSHV virions were prepared from iSLK-BAC16 cells and KSHV infection was determined by green fluorescent protein (GFP) in immortalized OKF6/TERT oral keratinocytes cultured in monolayer and 3-dimensional organotypic cultures. HIV EV RNA was determined by quantitative RT-PCR. **Results:** 1) EVs isolated from HIV+ patient significantly enhanced KSHV infectivity in monolayer and 3-D cultures of OKF6/TERT cells compared with those from saliva of healthy volunteers; 2) EV-depleted HIV+ patient saliva failed to stimulate KSHV infection and transmission; 3) HIV-associated saliva EVspromoted KSHV infectivity in oral epithelial cells in an epidermal growth factor receptor (EGFR)-dependent manner; 4) HIV trans-activation response (TAR) element RNA was responsible for HIV-associated saliva EV induced KSHV infection in oral epithelial cells. **Conclusion:** EVs derived from HIV+ patient saliva promote KSHV infection and transmission. The HIV TAR RNA is critical for stimulating KSHV infection and transmission in oral epithelial cells. Controlling release of the EVs or direct targeting of TAR may serve as a novel strategy for prevention of KSHV infection and transmission

A-384

Quantifying the Extent of Kappa Serum Free Light Chain Reagent Drift Using Lot to Lot Evaluation with Abnormal Patient Pools

S. Cotten, N. Korpi-Steiner. *Univ North Carolina, Chapel Hill, NC*

Serum free light chains (FLC) remain an important clinical laboratory test for diagnosis, prognosis, and management of treatment for patients with plasma cell disorders. Monitoring FLC values over time is especially important to evaluate a patient's response to therapy including novel therapeutics in clinical trials. The objective of the study was to quantify kappa and lambda serum free light chain reagent drift between lots over a 24 month period. The impact of different reagent lots for FLC was monitored for a period of 2 years using residual patient specimens measured on the current lot and single set of high abnormal patient pools for both kappa and lambda FLC. All FLC measurement were performed on the The Binding Site Optilite® instrument from 2017-2018. For every new lot or new lot shipment, 10 patient specimens and the high abnormal pool control was measured on the current lot in use and new lot under evaluation. Linear regression was performed with the paired results to generate slope values representing the performance of each new lot or shipment. 19 kappa evaluations were performed that covered 13 new (sub)lots and 6 lot shipments. 14 Lambda evaluations were performed that covered 10 new (sub)lots and 4 lot shipments. To evaluate the bias between lots, the deviation of the slope from 1.0 was calculated from the linear regression statistics. The cumulative slope deviation over time was defined as the sum of all lot deviations from 1.0 over the 24 month period. The criteria for acceptability of patient results in evaluating new lots was defined as +/- 3 mg/dL for results <15 mg/dL or +/- 20% for results above 15 mg/dL. Results of the study demonstrated that all patient specimens and patient pool results were within the acceptable criteria for allowable error in comparison to the previous lot. The analysis of individual slope deviations for lambda showed a maximum slope deviation of 0.135 from the previous lot. The analysis of individual slope deviations for kappa showed a maximum slope deviation of -0.2734 from the previous lot. When the cumulative slope bias over 24 months for lambda and kappa FLC reagents were compared they showed divergent performance. The cumulative slope deviation for lambda FLC reagent over 24 months was 0.1498. The cumulative slope deviation for kappa FLC was -0.9374 over the 24 month study period. The large decline in kappa FLC values over time was not detected by QC or with individual evaluation of new lots with 10 patient specimens and an abnormal high pool. The study suggests that acceptable criteria between lots for individual samples may not be suitable to detect subtle lot to lot drift over time. Application of linear regression and calculation of slope deviation over time between several lots can be used to evaluate reagent drift. This is particularly important for assays where commercial quality control does not appropriately span abnormal ranges frequently found in patient specimens.

A-385

A Preliminary Algorithm for the Early Diagnosis of Gastric Cancer by Combining Abbott Serum Tumor Biomarker Assays

X. Wang[1], X. Qin[2], Y. Sun[1], T. Sun[1], C. Lee[1], P. Yin[1]. *[1]Abbott Diagnostics Division, China R&D, Shanghai, China, [2]Peking Union Medical College Hospital, Clinical Laboratory, Bejing, China*

Background: Gastric cancer is an aggressive disease which remains one of the most common malignancies and the second leading cause of cancer-related deaths worldwide. Blood based tests including tumor markers like carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), CA12-5, pepsinogen 1 (PG1), pepsinogen 2 (PG2) and progastrin-releasing peptide (ProGRP) are quick and easy way for early diagnosis of gastric cancer, compared with endoscopic biopsy and histopathological evaluation for patients and hospitals. However, the performance of detection based on one single marker is deficient and contribute to a lower diagnostic value. Thus, finding a better algorithm by using multiple tumor markers are greatly needed for early diagnosis and treatment of gastric cancer patients.

Method: A total of 182 clinical defined gastric cancer patients and 210 healthy controls were recruited from Peking Union Medical College Hospital in China. Serum samples were collected and measured by ARCHITECT® systems. Eight bio-markers were selected for algorithm design including C-reactive protein (CRP), Ferritin (FER), PG1, PG2, CA12-5, ProGRP, CEA, and CA19-9 together with age and gender. The results were analyzed by using a logistic regression method. Receiver operating characteristic curve (ROC) was used to compare area under the curve between individual biomarker and the algorithm.

Results: The ROC results showed CRP (AUC= 0.661), FER (AUC = 0.587), PG1 (AUC = 0.659), PG2 (AUC = 0.77), CA12-5 (AUC = 0.511), ProGRP (AUC = 0.51), CEA (AUC = 0.70), and CA19-9 (AUC = 0.463). A statistically significant increase of AUC (0.91) was found in the developed algorithm that combined biomarkers, age and gender.

Conclusion: Our findings indicated that our new algorithm with combined biomarkers, age and gender might be a useful and practical way for early-stage gastric cancer detection.

Further studies are needed and tested with larger sample size in conjunction with other clinical methods to ensure the diagnostic value of newly developed algorithm.

A-386

Preliminary Evaluation of CA242 Assay on Fully-Automated Chemiluminescent Immunoassay Analyzer

J. Liu[1], S. Ning[1], X. Qiu[1], B. Tu[1], T. Leary[2], Z. Lin[2], M. Pope[2], P. Schultz[2], A. Narváez[2], C. Lee[1], P. Yin[1]. *[1]Abbott Diagnostics Division, China R&D, Shanghai, China, [2]Abbott Laboratories, Abbott Park, IL*

Background: CA 242 is a dual utility marker for colorectal and pancreatic cancers. It has lower sensitivity than CEA, but higher specificity in aiding diagnosis of colorectal cancer. CA242 has similar or lower sensitivity, but better specificity than CA19-9 in aiding diagnosis of pancreatic cancer. Although the detailed structure of CA242 epitope is not exactly known, it has been confirmed that CA242 is related, but chemically and immunologically distinct from CA19-9. In this study, a prototype ARCHITECT® CA242 assay has been developed and evaluated. It is designed for quantitative de-

tection of CA242 in serum on the fully-automated chemiluminescent immunoassay analyzer.

Materials and Methods: The ARCHITECT® CA242 prototype assay was developed for use with the ARCHITECT® Immunoassay i System. The key biologics were in-housing manufactured. The assay determines serum CA242 concentration using a two-step sandwich assay format. In the study, the analytical performances including the precision, limit of quantitation (LoQ), linearity, and interference studies were evaluated according to CLSI guidelines. Total of 101 clinical serum samples collected from major Chinese hospitals were analyzed and compared with a commercial CanAg CA242 EIA kit (Fujirebio, Sweden).

Results: The measuring intervals of ARCHITECT® CA242 prototype assay cover between 1.0 U/mL to 500 U/mL. Total imprecision, LoQ, linearity and other evaluation item results are shown for ARCHITECT® CA242 in the table below. Results versus on-market product demonstrated a slope 0.94 and R = 0.90.

Evaluation Item	Results
Total %CV	3-7 % CV
LoQ	0.03 U/mL
Linearity	1.0-500 U/mL
Cross-reactivity	Cross-reactivity with CA19-9 is observed (CA19-9 120 U/mL). Cross-reactivity for other tumor markers are not observed. (CA 125 3.85 KU/mL, CA 153 800 U/mL, CA 724 1000 U/mL, CEA 550 ng/mL, AFP 2100 ng/mL, hCG 600 mIU/mL, CYFRA21-1 1100 ng/mL)
Interference	Interference is not observed; (Bilirubin, Hemoglobin, Protein, Triglyceride, Biotin)
HAMA interference	HAMA interference is not observed
Method Comparison	Slope=0.94, R=0.90 (N=101, vs CanAg CA242 EIA)

Conclusion: The ARCHITECT® CA242 prototype assay performs an extended working range and good precision, reproducibility and sensitivity. Excellent correlation of results is observed between ARCHITECT® and a commercial kit. It provides the fast & accurate detection of CA242 in serum and can be used as an aid in the clinical evaluation of symptomatic patients suspected of having pancreatic cancer, colorectal and other related diseases.

A-387

Detection of Lung Cancer by Breath Analysis with Chemoselective Microreactors

Z. Xie, Q. Li, T. Sibakoti, M. Nantz, V. van Berkel, X. Fu. *University of Louisville, Louisville, KY*

Background: The objective of this study is to analyze exhaled breath with chemoselective microreactors for detection of early lung cancer. The analysis of exhaled breath is a promising noninvasive approach for detection of early lung cancer. Lung cancer continues to be the leading cause of death among all cancers for men and women. One of the crucial factors in the fatality rate of lung cancers is the lack of early detection tools. This study reports quantitative analysis of carbonyl volatile organic compounds in exhaled breath by a novel chemoselective microreactor approach and identification of specific lung cancer related volatile metabolites which can accurately differentiate benign from malignant pulmonary nodules.

Methods: Silicon microreactor chips were fabricated using microelectromechanical system (MEMS) fabrication techniques. The microreactor chips have a microfluidic channel volume of 20 microliter. There are thousands of micropillars in the microreactor coated with 2-(aminooxy)-N,N,N-trimethylethanammonium (ATM) iodide. The ATM functionalized micropillars distribute breath sample flow and capture carbonyl VOCs by means of oximation reactions. After flowing breath sample through the microreactor, ATM-carbonyl adducts are eluted from the chip with methanol and analyzed by ultra high performance liquid chromatography-mass spectrometry (UHPLC-MS). Alveolar breath sample of 420 mL after exclusion of the first 650 mL of tidal breath were collected from 50 healthy controls, 65 patients with early stage lung cancer (stage I and II) and 27 patients with benign pulmonary nodules. The concentrations of carbonyl compounds in exhaled breath samples were calculated based on quantitative analysis by UHPLC-MS.

Results: A panel of four carbonyl compounds including hydroxyl-acetaldehyde, 2-butanone, 3-hydroxy-2-butanone and decanal have been identified to have significantly higher concentration (p value<0.05) in exhaled breath of lung cancer patients than that in exhaled breath of patients with benign pulmonary nodules and healthy controls. Threshold concentrations of these four carbonyl compounds as one standard deviation above the average concentration of the healthy controls. The sensitivity of predication of lung cancer achieves 92% if lung cancer patients by at least one compound above its threshold concentration. The specificity of predication of benign pulmonary nodules achieves 85%. The elevated carbonyl concentrations in lung cancer patients decreased to the ranges of healthy controls after resection.

Conclusion: The analysis of carbonyl VOCs in exhaled breath can be used as an adjunct to computed tomography (CT) for the diagnosis of lung cancer in its early stages and can distinguish benign from malignant nodules.

Tuesday, August 6, 2019

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

A-389

High Prevalence of Chronic Hepatitis Delta Virus Infection in Eastern Mongolia

N. Biziya[1], B. Nyamaa[2], D. Enkhbayar[1]. *[1]Molor laboratory, Choibalsan, Mongolia, [2]Mongolian National University of Medical Science, Ulaanbaatar, Mongolia*

Background: Hepatitis delta virus (HDV) is a serious cause of liver-related morbidity and mortality worldwide. The aim of this study is to determine the prevalence of HDV infection among patients positive for hepatitis B surface antigen (HBsAg) living in the Dornod province, in Eastern Mongolia.

Methods: We retrospectively analyzed the data obtained from 121 consecutive patients with HBV infection, who were referred to Dornod Medical center between 2017 and 2018. Liver transaminases were analyzed using a commercial biochemistry kit. The presence of HBV and HDV infection was investigated using both ELISA and polymerase chain reaction (PCR) methods. Anti-HDV-positive individuals were examined to determine HDV-RNA level by PCR.

Results: Of all patients, 53 were men (44.9%) & 68 were women (56.1%). The average age was 44 (between 16 and 70 years). Anti-HDV was positive in 75% (91/121) and all were checked for HDV RNA and 100% were found positive (91/91). HBV-DNA was detected in 111 of the 121 patients. Mean HBV-DNA level were 1,590,177.877 IU/ml. The mean HBV-DNA level in the anti-HDV-positive patients was significantly lower than in the anti-HDV-negative patients ($P < 0.001$). There were 65 patients with cirrhosis (54%) in the study group. Anti-HDV seroprevalence and HDV RNA presence were higher in those with cirrhosis 42 (64%). HDV-RNA-positive patients had significantly higher ALT (94 U/L) levels when compared to HDV-RNA-negative patients.

Conclusions: Our study showed HDV infection in HBsAg positive patients who live in Eastern province Mongolia higher than other provinces of Mongolia.

A-391

Traditional Markers Combined with HBcrAg, pg-RNA to Supervise the Therapeutic Effect about HBeAg-positive Patients with Antiviral Therapy

B. Lou, G. Ma, F. Lv, D. Zhao, Z. Zhang, Y. Chen. *First Affiliated Hospital, College of Medicine, Zhejiang University, Hang Zhou, China*

Background: 350 million people are chronically infected with hepatitis B virus (HBV) worldwide. We aimed to use the serum hepatitis B core-related antigens (HBcrAg), hepatitis B surface antigen (HBsAg), pg-RNA (pregenome RNA) and other viral markers to evaluate the therapeutic effect of patients with anti-viral therapy.

Methods: 71 HBeAg-positive patients received anti-viral therapy between 2013 to 2016 were enrolled in the First Affiliated Hospital of Zhejiang University School of Medicine, then detected the levels of serum HBcrAg, pg-RAN, HBsAg, HBeAg, ALT and HBV-DNA at different times. Also, we define the decreased titer of HBcrAg and HBsAg from baseline to 6 months and baseline to 12 months as ΔHBcrAg and ΔHBsAg, meanwhile, we use the ΔHBcrAg and ΔHBsAg to predict HBeAg seroconversion in HBeAg-positive patients. The predictive power of on-treatment levels of ΔHBsAg and ΔHBcrAg was determined using receiver operating curve (ROC) analysis and cut-off values determined by maximized Youden's index.

Results: About 35.2% of patients achieved HBeAg seroconversion after a median of 29 months' treatment. Longitudinal analysis of samples from baseline, 6 months of anti-viral treatment demonstrated significant differences in the kinetics of HBcrAg, HBsAg, HBV DNA, HBeAg and pg-RNA between the HBeAg seroconverters and nonseroconverters ($P<0.05$). Nevertheless from 6 months to 12 months, their differences were not as significant as baseline to 6 months except HBV DNA ($P=0.0224$). Using ROC to predict the seroconversion of HBeAg: at 6 months, ΔHBcrAg of 0.85 log10 IU/mL and ΔHBsAg of 0.39 log10 IU/mL has the maximized Youden's index with AUC of 0.724 (0.597, 0.850; 95%CI) and 0.668 (0.536, 0.800; 95%CI). At 12 months, ΔHBcrAg of 2.05 log10 IU/mL and ΔHBsAg of 0.81 log10 IU/mL has the maximized Youden's index with AUC of 0.707 (0.569, 0.845; 95%CI) and 0.660 (0.519, 0.800; 95%CI). Combination of ΔHBcrAg and ΔHBsAg at these time-points had a stronger predictor of HBeAg seroconversion. The ΔHBcrAg and ΔHBsAg was greater than 0.90 and 0.52 log10 IU/mL at 6 months, which were demonstrated as predictor of HBeAg seroconversion. At 12 months, combination of ΔHBcrAg 1.90 log 10 IU/mL and ΔHBsAg 0.50 log10 IU/mL was selected as predictor with an appropriate sensitivity and specificity.

Conclusion: Our results may be used to identify patients who can easily achieve HBeAg seroconversion, that would help to decide the time of treatment end-points.

A-392

HBcrAgand pg-RNA Combined with Traditional Markers to Supervise the Seroconversion of HBsAg with Anti-viral Therapy

G. Ma, Y. Chen. *Department of Laboratory Medicine, First Affiliated Hospital, College of Medicine, Zhejiang Universi, Hangzhou, China*

Objective: In this retrospective analysis, we aimed to identify predictor of seroconversion using serum quantitative pg-RNA, HBcrAg and HBsAg, in CHB patients treated with nucleos(t)ide analogues(NA) or PEG-IFN.

Method: A total of 335 patients started on antiviral therapy between 2013 to 2017 were enrolled in the First Affiliated Hospital of Zhejiang University School of Medicine, in these patients, only 18 achieved the seroconversion of HBsAg, and other 84 no seroconversion of HBsAg patients were selected randomly in 317 patients. Then the samples and date of 102 patients were retrospectively analyzed at different time. Also, we define the decreased titer of pg RNA, HBcrAg and HBsAg from baseline to 6 months and baseline to 12 months as Δpg RNA, ΔHBcrAg and ΔHBsAg, meanwhile, we use the Δpg RNA, ΔHBcrAg and ΔHBsAg to predict HBsAg seroconversion in HBsAg-positive patients. The predictive power of on-treatment levels of Δpg-RNA, ΔHBcrAg and ΔHBsAg was determined using receiver operating curve(ROC) analysis and cut-off values determined by maximized Youden' index.

Result: About 5.4% of patients achieved HBsAg seroconversion after a median of 3.61 years' treatment. Using ROC to predict the seroconversion of HBsAg: at 6 months, ΔHBsAg of 0.64 log_{10} IU/mL has the maximized Youden's index with AUC of 0.886 (0.802, 0.969; 95%CI). At 12 months, ΔHBsAg of 1.45 log10 IU/mL has the maximized Youden's index with AUC of 0.939 (0.868, 1.000; 95%CI). Longitudinal analysis of samples from baseline, 3 and 6 months of anti-viral treatment demonstrated significant differences in the kinetics of HBcrAg, HBsAg, HBV DNA and pg-RNA between the HBsAg seroconverters and nonseroconverters ($p<0.05$) except HBV DNA ($p=0.188$) from 3 months to 6 months. From 6 months to 12 months, their differences were also significant except HBV DNA($p=0.993$) and pg RNA ($p=0.426$). The comparison of HBcrAg "conversion" rates using Kaplan-Meier method between 18 patients with HBsAg conversion and 84 patients with HBsAg no conversion indicate that the two group have significant difference at the time of antiviral discontinuation ($p=0.0124$).

Conclusion: The seroconversion of HBsAg is the ultimate goal in the patients with chronic hepatitis B (CHB). While the purpose of elimination of HBsAg is very difficult and the rate of seroconversion is poor using current antiviral treatment. According to our result, we can use HBsAg, HBcrAg and pg RNA to pick out the appropriate patients who have the potential to achieve seroconversion by sticking to antiviral therapy is very important to reach the target of functional cure or even clinical cure.

A-393

TRIM22, TRIM26 and ZNF350 Polymorphisms are Associated with Different Clinical Outcomes of Interferon-átreated CHB Patients

Q. Ou, J. Guo, S. Guo. *The department of medical laboratory science, Fuzhou, Fujian Province, China*

Background: To investigate the impact of gene polymorphisms in predicting interferon-αresponse in patients with HBV infection.

Methods: Single nucleotide polymorphisms were examined in 124 patients with chronic HBV infection using Infinium® Asian Screening Array. SNPs and haplotypes were identified after adjustment for age, sex, HBV DNA.

Results: The allele association analysis revealed that the TRIM22 rs10838543 (G vs A), TRIM26 rs12175655 (G vs A) and ZNF350 rs2278420 (G vs A) markers significantly affected the achievement of complete response (CR) (P = 0.012, OR = 2.548, 95%CI: 1.208-5.373; P= 0.003, OR = 2.684, 95%CI: 1.391-5.177; $P=10^{-5}$, OR=0.180, 95%CI: 0.076-0.430, respectively). However, in the genotype analysis, only TRIM26 rs12175655 and ZNF350 rs2278420 was significantly associated with

CR (GG vs AG+AA, P = 0.004, OR = 3.173, 95%CI: 1.421-7.086; AA vs GG+GA, P=10^{-4}, OR=6.016, 95%CI: 2.591-13.968, respectively).The association analysis for partial response (PR) showed that the AA genotype of TRIM22 rs10838543 and the AA genotype of ZNF350 rs2278420 was significantly more frequent (P = 0.031, OR = 0.399, 95%CI: 0.186-0.828; P=10^{-4}, OR=0.180, 95%CI: 0.089-0.364, respectively).

Conclusion: Our results suggest that TRIM22, TRIM26 and ZNF350 polymorphisms are associated with different clinical outcomes in CHB patients treated with interferon-α.

A-394

Characterization and Potential Clinical Significance of Natural Variability in Hepatitis B Virus Reverse Transcriptase among Treatment-naive Chinese Patients

Q. Ou, Y. Fu. *The department of medical laboratory science, Fuzhou, Fujian Province, China*

Background: Mutations in HBV RT are associated with nucleos(t)ide(s) analogue resistance, which happen during the long-term antiviral treatment. However, the characterization of mutations in HBV RT among treatment-naïve Chinese patients and its clinical significance are still ambiguous. **Objective:** To investigate the characterization and potential clinical significance of natural variability in HBV RT among treatment-naïve Chinese patients with CHB. **Methods:** HBV RT sequences from rt145 to rt289 were amplified and sequenced among 427 treatment-naïve Chinese patients with CHB. **Results:** 12 AA sites were identified as B- and C-genotype-dependent polymorphic AA positions. No classical primary or secondary NAr mutations were detected except rtA181T. Potential NAr mutations were found in 36.53% patients involved in 55 RT AA sites, among which concomitant AA changes occurred in 26 AA sites of S gene. Mutation clusters in different sections of HBV RT region were diverse among HC group, CHB group and ALD group. Patients with multiple mutations showed signifcantly decreased serum HBV DNA loads, the mean level of serum HBsAg, the HBeAg-positive ratio but increased average age. The presence of multiple RT mutant sites was significantly associated with severity of liver fibrosis. **Conclusion:** The analysis of genotype-dependent AA polymorphic positions and potential NAr mutations in RT region among different liver disease stages might provide a possible explanation to illuminate the mechanisms of the evolution and selection under the varying immune pressure. The correlation between RT mutations and clinical characterizations was discussed to emphasize the overall effect of natural occurring RT mutations on untreated patients.

A-395

Large Imprecision Near Cutoff Level Complicates Interpretation of Low Positive Samples by the Quantiferon-TB Gold-Plus Assay

X. Zhang, L. Reed, T. Daly. *Cleveland Clinic, Cleveland, OH*

Background: Interferon gamma release assays are important tools in identifying both active and latent tuberculosis infection. Recently, the QuantiFERON-TB Gold-Plus (QFT-Plus) replaced the QuantiFERON-TB Gold In-Tube (QFT-GIT) in many clinical laboratories. QFT-Plus utilizes two antigen tubes to elicit an immune response from CD4+ and CD8+ T-cells. A number of published studies evaluated the clinical performance of the QFT-Plus and revealed some discordance between the two assays, especially for low positive samples. However, data regarding the analytical performance of the assay is scarce. Knowing the analytical performance is important for providing insight into the possible sources of discrepancy and to aid result interpretation.

Methods: QFT-Plus assay was performed on three automated ELISA instruments (DSX; Dynex Technologies). Healthy donors, healthcare workers and patient samples were used in the study. The assay precision, accuracy, sensitivity and linearity were evaluated, and data was analyzed using EP Evaluator software.

Results: QFT-Plus assay showed acceptable performance (Figure 1) in most parameters. However, imprecision was about 40% for low positive samples. Method comparison with a reference laboratory using split post-incubation plasma samples showed comparable analytical performance and 97.5% agreement in result interpretation (positive, negative and indeterminate). One borderline sample was negative by the reference lab but positive by our lab. Furthermore, positive rate was 2.16% (n=1948) after implementing the QFT-Plus which was similar to the QFT-GIT (3.4%; n=16642). TB1 or TB2 single tube positive samples were account for 19.0% and 21.4% of the total positives respectively and all with a value <0.7 IU/mL categorizing as low positive. Additional 26.2% low positives were positive for both TB1 and TB2.

Conclusion: The analytical performance of the QFT-Plus assay is acceptable. However, low positive results should be interpreted with cautious due to the large imprecision near cutoff level.

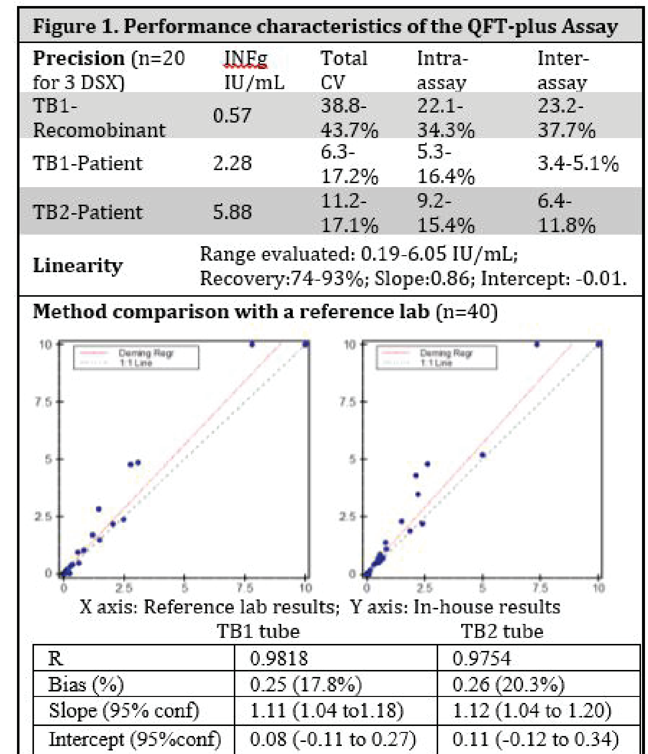


Figure 1. Performance characteristics of the QFT-plus Assay

Precision (n=20 for 3 DSX)	INFg IU/mL	Total CV	Intra-assay	Inter-assay
TB1-Recomobinant	0.57	38.8-43.7%	22.1-34.3%	23.2-37.7%
TB1-Patient	2.28	6.3-17.2%	5.3-16.4%	3.4-5.1%
TB2-Patient	5.88	11.2-17.1%	9.2-15.4%	6.4-11.8%
Linearity	Range evaluated: 0.19-6.05 IU/mL; Recovery:74-93%; Slope:0.86; Intercept: -0.01.			

Method comparison with a reference lab (n=40)

	TB1 tube	TB2 tube
R	0.9818	0.9754
Bias (%)	0.25 (17.8%)	0.26 (20.3%)
Slope (95% conf)	1.11 (1.04 to1.18)	1.12 (1.04 to 1.20)
Intercept (95%conf)	0.08 (-0.11 to 0.27)	0.11 (-0.12 to 0.34)

A-396

Performance Evaluation of the ADVIA Centaur Quantitative HBsAg Assay

A. Schroff, B. Healy, S. Lue, M. Anthony, S. Patibandla. *Siemens, Tarrytown, NY*

Background: Hepatitis B virus (HBV) is endemic throughout the world and is the major cause of liver disease. Hepatitis B surface antigen (HBsAg) is a distinctive serological marker of acute or chronic hepatitis B infection. Serum HBsAg levels are inversely correlated with the control of the infection: the higher the infection control, the lower the serum HBsAg level. We report the analytical performance of a new quantitative assay for HBsAg (QHBs) on the ADVIA Centaur® Immunoassay System.

Methods: The LoB, LoD, and LoQ of the ADVIA Centaur QHBs assay were evaluated by testing 5 HBsAg-negative and 10 low-positive samples per CLSI document EP17-A2. Accuracy was assessed versus the WHO 3rd International Standard (12/226). High-dose hook effect was evaluated up to a concentration of 3.5 mg/mL of HBsAg. Linearity was evaluated per CLSI document EP6-A. Precision was determined using a 20-day protocol employing two systems and two runs per day. HBsAg results are reported in IU/mL, with an assay range for undiluted samples of the LoQ to 125 IU/mL.

Results: The ADVIA Centaur QHBs assay was determined to have an LoB of 0.006 IU/mL, an LoD of 0.012 IU/mL, and an LoQ of 0.019 IU/mL. No hook effect was observed up to a concentration of 3.5 IU/mL. The assay was linear across the reportable range of 0.020 IU/mL to 125 IU/mL. Extended range was achieved through the use of automated onboard dilutions of 1:500 and 1:2500, with 85% of HBsAg-positive samples reading inside the assay range at a 1:500 dilution. The QHBs assay had a total %CV of <8% across the extended assay range over a 20-day period with three lots of reagents.

Conclusions: The results of these studies show state-of-the-art performance for the fully automated ADVIA Centaur QHBs assay.

*Product availability will vary by country. Not available for sale in the U.S.

A-397

A Fully Automated Sample-to-Answer PCR System for Easy and Sensitive Detection of *Clostridium difficile* in Human Stool

P. A. Lee[1], M. Lu[2], Y. Lin[1], C. Tai[1], Y. Tsai[1], H. G. Chang[1], H. Tang[3]. [1]*GeneReach Biotechnology, Taichung, Taiwan,* [2]*Division of Infectious Diseases, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan,* [3]*Department of Microbiology and Immunology, School of Medicine, China Medical University, Taichung, Taiwan, Taichung, Taiwan*

Background: *Clostridium difficile* (*CD*) infections have been associated with 15-25% of antibiotic-associated diarrhea, resulting in huge health care-related costs worldwide. Nucleic acid testing (NAT) is easier and faster than toxigenic culture and has better sensitivity than immunoassay and enzyme testing algorithms. Based on the insulated isothermal PCR (iiPCR) technology, the POCKIT Central *C. difficile* reagent works with the compact, automated, sample-in-answer-out CE-marked POCKIT Central Nucleic Acid Analyser to allow easy and fast NAT near patients, minimizing human error risks and providing timely results to help patient care and reduce hospital costs. Here, we aimed to evaluate the analytical and clinical performance of the POCKIT Central *C. difficile* system, which detects the *toxin B* (*tcdB*) gene, an essential gene for *CD* infection, and includes an internal control to monitor both NA extraction and PCR. **Methods:** After being treated in Lysis Mix at 37°C for 10 min and at 65°C for 10 min, stool mixture was placed into the first well of a preloaded Extraction Cartridge, which was subsequently placed into POCKIT Central for automated NA extraction and PCR detection. Limit of detection (LoD) and precision were evaluated using *CD*-negative stools spiked with a toxigenic *CD* strain of known titers (CFU/ml). CD-negative stools spiked with different CD strains, relevant pathogens, and interference substances were tested to evaluate inclusivity, exclusivity, and interference, respectively. Clinical performance of the index *CD* system was evaluated by side-by-side comparison with that of a CE-marked real-time PCR (qRT-PCR) system (PSP Spin Stool DNA Kit and RealStar *Clostridium difficile* PCR Kit 1.0) with 107 stool samples. **Results:** Tested with serial dilutions of the *CD*-spiked stool, the index *CD* PCR system was calculated by probit test to have a $LoD_{95\%}$ of 499 CFU/ml. The index system did not react with one non-toxigenic *CD* strain and eight other bacteria, indicating good specificity. The index reagent detected *CD* in low positive samples in the presence of 11 potential interference substances. Testing low and moderate positive samples showed excellent repeatability among repeated tests for 12 days, and excellent reproducibility among repeated tests by two operators at two sites for 5 days. In clinical performance, 52 and 55 samples were positive and negative by the qRT-PCR assay, respectively; four of the qRT-PCR-positive samples were negative by the index system and two of the qRT-PCR-negative samples were positive by the index system. Repeated testing of the discrepant samples suggested that most were of low *CD* titers. A kappa analysis of the results found a high inter-rater agreement (94.39%; $CI_{95\%}$, 89.51 - 99.27%; κ = 0.89) between the two PCR systems, with a positive agreement of 92.31% (CI95%, 83.94 - 100%) and negative agreement of 96.39% (CI95%, 89.77 - 100%). **Conclusions:** With great analytical performances and clinical performance comparable to those of the reference qRT-PCR system, the POCKIT Central *C. difficile* reagent on the compact automated PCR system can serve as an easy and fast NAT tool to aid the diagnosis of *CD* infection.

A-399

Urine Sediment Analyzers Based on Poisson Distribution Theory - Precision of Atellica UAS 800 and IQ200

K. Das, M. Lynch, C. Tilghman. *Siemens Healthineers, Norwood, MA*

Background: Various distribution theories are applied to predict probability of events occurring under different conditions. Normal (Gaussian) distribution is commonly used to predict the probability outcomes where analyte concentrations are measured with continuous numerical output. However, the analyzers which count discrete events rather than measuring a changing signal are appropriately estimated using the Poisson distribution. The Poisson distribution is applicable when the events occur: (1) in discrete numbers, (2) in independent manner, (3) at a constant rate and (4) with proportionally increasing probability with increasing interval. The theoretical counting errors inherent in the Atellica® UAS 800* and IQ200 image based analyzers are governed by Poisson Distribution theory. In this poster, characteristics of these analyzers relevant to Poisson distribution and the calculation of theoretical repeatability Coefficient of Variation (CV) are discussed. The repeatability CVs of these analyzers are determined under same experimental conditions and compared with theoretical-CVs. The results are compared to observed results.

Methods: DIP&SPIN (Quantimetrix, CA) urine quality control materials were analyzed for 20 days with 2 runs per day in 2 replicates on Atellica UAS 800 (Siemens Healthineers) and IQ200 (Beckman Coulter) analyzers. Observed repeatability CVs were determined. The theoretical-CVs were estimated as the Poisson counting error using the relationship: $CV_{theoretical} = 1\times100/\sqrt{(\text{total count})}$. The ratios between observed-CVs and theoretical-CVs were calculated and compared with literature reported ratios.

Results: The observed-CVs and theoretical-CVs are in the table below.

Conclusion: The results indicated that the observed/theoretical CV ratios for IQ200 and Atellica UAS800 analyzers vary from 0.91 to1.66. Similar ratios (0.73-2.22) can be estimated from reported IQ200 precisions (Wah DT *et. al.* Clin Chem, 2005, 123: 290-296). The ratios' departure from 1 were perhaps due to pre-analytical variations. The ratios indicate the repeatability precision of these analyzers can be predicted using Poisson distribution theory at a given concentration. *Not available for sale in U.S.A.

The theoretical-CVs, the observed-CVs and their ratios for Atellica UAS 800 and IQ200

QC Materials (DS = DIP&SPIN)	Analyzer	Analyte	N	Concentration (p/µL)	Theoretical Repeatability CV (%)	Observed Repeatability CV (%)	Ratio (Obs./The.)
DS1-Level 1	IQ200	RBC	64	6.7	27.3	45.3	1.66
DS1-Level 1	Atellica UAS 800	RBC	59	12.1	26.7	27.9	1.03
DS1-Level 1	IQ200	WBC	64	14.1	18.9	22.1	1.17
DS1-Level 1	Atellica UAS 800	WBC	59	8.5	27.7	31.0	1.19
DS1-Level 2	IQ200	RBC	64	60.3	9.1	11.9	1.31
DS1-Level 2	Atellica UAS 800	RBC	60	84.5	10.2	14.4	1.41
DS1-Level 2	IQ200	WBC	64	73.0	8.3	8.8	1.06
DS1-Level 2	Atellica UAS 800	WBC	60	62.0	10.3	10.5	1.02
DS2-Level 1	Atellica UAS 800	RBC	80	11.7	27.4	25.2	0.92
DS2-Level 1	Atellica UAS 800	WBC	80	11.4	24.1	28.0	1.16
DS2-Level 2	Atellica UAS 800	RBC	80	70.2	11.2	13.6	1.21
DS2-Level 2	Atellica UAS 800	WBC	80	58.8	10.6	11.7	1.10

A-400

A de novo, Specific Dengue Virus Detection Method using Ultrasensitive ELISA

E. Ito[1], M. Awano[1], K. Nakaishi[2], S. Watabe[2], T. Yoshimura[3]. [1]*Waseda University, Tokyo, Japan,* [2]*TAUNS Laboratories, Inc., Shizuoka, Japan,* [3]*Health Sciences University of Hokkaido, Hokkaido, Japan*

Background: Dengue virus (DENV) causes dengue fever, and it belongs to a genus *Flavivirus*, which includes West Nile virus, tick-borne encephalitis virus, yellow fever virus, Zika virus and so on. WHO suggested that for mid- to long-term DENV tests, the detection of immune-complex dissociated NS1 antigen was best in a rapid test format (https://www.who.int/tdr/publications/documents/dengue_diagnostics.pdf). However, the need to distinguish DENV from the other flaviviruses has been strongly emphasized. In other words, we could not long use the specific antibodies for DENV NS1. Recently, we had a chance to use the monoclonal antibodies for DENV NS1, which do not cross-react against other flavivirus NS1. In the present study, we developed an ultrasensitive ELISA coupled with thio-NAD cycling, using the two DENV NS1 antibodies, to produce a de novo, specific DENV detection kit.

Methods: A sandwich method using a primary and a secondary antibody (Bio Matrix Research, Chiba, Japan) for DENV NS1 antigens was employed in our ultrasensitive ELISA. An androsterone derivative, 3α-hydroxysteroid, was produced by the hydrolysis of 3α-hydroxysteroid 3-phosphate with alkaline phosphatase linked to the secondary antibody. This 3α-hydroxysteroid was oxidized to a 3-ketosteroid by 3α-hydroxysteroid dehydrogenase (3α-HSD) with a cofactor thio-NAD. By the opposite reaction, the 3-ketosteroid was reduced to a 3α-hydroxysteroid by 3α-HSD with a cofactor NADH. During this cycling reaction, thio-NADH accumulated in a triangular-number fashion. Accumulated thio-NADH was measured directly at an absorbance of 405 nm without any interference from other cofactors.

Results: When we made a calibration curve line using standard NS1 antigen, the limit of detection (LOD) was 6.12×10^{-18} moles/assay, and the limit of quantification (LOQ) was 2.04×10^{-17} moles/assay. Here, we used the formula of LOD=3 SD/Slope and LOQ=10 SD/Slope, where SD is the standard deviation from the blank measurement, and Slope from the calibration curve line of the standard. Our result of LOD, 6.12×10^{-18} moles/assay, means 2.45 pg/mL. This value is at least 2 orders of magnitude more sensitive than that of one of commercially available kits, which detects 500 pg/mL of DENV NS1. Furthermore, spike-and-recovery tests using serum, urine and saliva confirmed the reliability of our ultrasensitive ELISA.

Conclusion: We developed an ultrasensitive ELISA to provide a diagnosis kit for DENV. The antibodies against DENV NS1 antigen were specific. Our system can detect attomole levels of NS1 not only in serum but also in urine and saliva. That is, this assay may offer a noninvasive test, thus reducing the patient burden.

A-401

Induction Heating: An Advanced Washing Technology to Preserve Sample Integrity on Alinity i and ARCHITECT i2000SR for Transfer to NAT Instruments

M. Effinger[1], A. Olivo[1], C. Williams[1], R. Whitlatch[1], A. Fischer[2], M. Rodgers[1], P. Soni[1]. [1]*Abbott Laboratories, Abbott Park, IL,* [2]*Abbott Laboratories, Irving, TX*

Background: In diagnostics laboratories, samples may be transferred between automated serological and molecular instruments, and the potential for sample contamination is a serious risk to the integrity of nucleic acid testing (NAT) results. In particular, the sensitive limit of detection (LOD) for HBV NAT assays combined with the high HBV titers encountered in specimens from patients with acute HBV infections presents a challenge for maintaining the sample integrity of negative specimens. Single-use pipette tips with filter barriers are commonly used to reduce this risk, but this increases the cost per test.

Aims: We evaluate the efficacy of applying induction heated washes to a non-disposable pipettor on serology instruments like the Abbott Alinity i and ARCHITECT i2000SR to preserve the integrity of samples transferred to downstream molecular testing instruments like the Abbott m2000 RealTi*me* system, which amplify nucleic acid targets exponentially.

Methods: In this application of induction heating, the metallic pipettor warms under its own resistance to coil-induced electrical currents. By sweeping the pipettor through an induction coil, temperatures on the pipettor are elevated throughout its length. Single donor high viral titer HBV genotypes A (8.98 log IU/ml), B (9.08 log IU/ml), and C (8.93 log IU/ml) specimens, and pooled donor high viral titer HBV genotype E (>7.83 log IU/ml) specimens were used as potential sources of contamination. The testing schema consisted of 4 testing blocks repeated 11 times on each instrument. Each testing block began with one high viral titer HBV specimen followed by three susceptible negative samples (HBV DNA negative human plasma, Abbott Molecular Diagnostics). Induction heated washes occurred between all samples processed on the serology instrument (Alinity i or ARCHITECT i2000SR, Abbott Diagnostics). The first susceptible negative sample tube in each block, with approximately 1.7 ml of residual sample volume, was then tested using the 0.5 ml Abbott RealTi*me* HBV assay with a limit of detection of 10 IU DNA/ml. An adverse event was defined as any susceptible negative sample which had a detectable level of HBV DNA.

Results: All first susceptible negative samples in each testing block (n = 44 per platform) run on Alinity i and ARCHITECT i2000SR using induction heated washes after a high viral titer HBV specimen reported no detectable level of the HBV DNA target (< 10 IU DNA/ml).

Summary/Conclusions: The integrity of samples tested on the Alinity i and ARCHITECT i2000SR for downstream molecular testing was preserved through the use of induction heated washes.

A-402

Identification of the First Human Isolate of Shewanella Haliotis Infection in the Western Hemisphere by Sequencing 16S rRNA Gene

D. Liu[1], V. Singh[1], L. Wang[2], R. HurtadoFiel[1], J. Pilz[1], M. Memmer[1], J. Y. Choi[1], E. Konadu[1], G. Pipia[1], D. H. Jokisch[1], C. C. Chen[1], G. Cheng[2], W. H. Rodgers* *Corresponding author[1]. [1]*New York Presbyterian Queens, Flushing, NY,* [2]*Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, CA*

Background: Shewanella haliotis is an emerging human pathogenic bacterium. Reports of Shewanella diseases have been increasing worldwide; including SSTI, bacteremia, gastroenteritis, and occasionally death. Each Shewanella species has different virulence and antibiotic susceptibility. Correct laboratory identification is important for targeted antimicrobial therapy and patient care. Currently there is no commercial automated ID system or MALDI-ToF that can identify S. haliotis. Using 16S rRNA gene sequencing methodology, we identified the first clinical isolate of S. haliotis from pelvic infection in the Western Hemisphere.

Methods: A pelvic abscess specimen was inoculated on plates of Sheep blood agar, Chocolate agar, and MacKoney agar. The isolate was subjected to biochemical analysis, antibiotic susceptibility, and genomic DNA sequencing.

Results: 1. Phenotypic features The isolate grew as 1-2 mm yellowish-brown mucoid colonies on the plates at 37⁰C and 42⁰C. β-hemolysis was observed on BAP. Gram stain revealed negative rods.

2. Biochemical features

Tests	Result	Tests	Result	Tests	Result
4mu-N-Acetyl-Bd-Glucos Aminide	+	D-Maltose	-	L-Lactate Alkalinisation	+
5-Keto-D-Gluconate	-	D-Mannitol	-	L-Leucine-Amc	+
Acetate	+	D-Mannose	-	L-Malate Assimilation	-
Adonitol	-	D-Sorbitol	-	L-Phenylalanine-Amc	+
Ala-Phe-Pro-Arylamidase	+	D-Tagatose	-	L-Proline Arylamidase	+
Alpha-Galactosidase	-	D-Trehalose	-	L-Proline-Amc	+
Alpha-Glucosidase	-	Ellman	-	L-Proline-NA	+
Alpha-Ketoglutaric Acid	+	Fermentation/Glucose	-	L-Pyrrolydonyl-Arylamidase	+
Arginine-Arginine-Amc	+	Gamma-Glutamyl-Transferase	+	L-Tryptophan-Amc	+
Beta-Alanine Arylamidase pNA	-	Glu-Gly-Arg-Arylamidase	+	Lysine Decarboxylase	-
Beta-Galactosidase	-	Glutamyl Arylamidase pNA	+	Lysine-Alanine-Amc	+
Beta-Glucoronidase	-	Glutaryl-Glycine-Arginine-AMC	+	Malonate	-
Beta-Glucosidase	-	Glycine Arylamidase	-	O/129 Resistance (Comp. Vibrio.)	-
Beta-N-Acetyl-Galactosaminidase	+	Glycine-Amc	+	Ornithine Decarboxylase	-
Beta-N-Acetyl-Glucosaminidase	+	Glycine-Proline-Amc	+	Oxidase	+
Beta-Xylosidase	-	H2S Production	+	Palatinose	-
Catalase	+	L-Arabitol	-	Phosphatase	+
Citrate(Sodium)	-	L-Glutamic Acid-Amc	+	Saccharose/Sucrose	-
Coumarate	-	L-Histidine Assimilation	-	Succinate Alkalinisation	+
D-Cellobiose	-	Lipase	-	Tyrosine Arylamidase	+
D-Glucose	-	L-Lactate Assimilation	+	Urease	-

3. Antibiotic susceptibility testing Vitek2 and Phoenix 100 were used to quantitatively determine minimal inhibition concentration. The isolate was sensitive to 14 antibiotics (Amikacin, Cefepime, Ceftazidime, Ceftriaxone, Gentamicin, Imipenem, Levofloxacin, Meropenem, Piperacillin-Tazobactam, Polymycin B, Tetracycline, Tigecycline, Tobramycin, Trimethoprim-Sulfamethoxazole) and resistant to 6 antibiotics (Ampicillin, Ampicillin-Sulbactam, Aztreonam, Cefazolin, Cefoxitin, Nitrofurantoin).

4. Genotype identification Querying the DNA sequence of this clinical isolate 16S rRNA gene in the GenBank database shows 99% homology to S. haliotis strain DW01. Phylogenetic analysis indicates that S. haliotis strain DW01 is one of the most recent ancestors of our clinical isolate.

Conclusion: The conventional identification methods based on phenotypic and biochemical characteristics have major limitations to some bacterial species, such as S. haliotis. Comparison of the bacterial 16S rRNA gene sequence has become an important laboratory diagnostic tool. 16S rRNA gene sequence analysis can identify rarely isolated, uncultivable, phenotypically uncharacteristic, slow growing species. Also it can discover novel pathogens.

DL, VS, LW, RH contributed equally

A-403

Fecal Calprotectin-Early Predictor of Relapse in Pediatric Inflammatory Bowel Disease in Indian Population

G. DEEPIKA, S. K, D. D. *ASIAN INSTITUTE OF GASTROENTEROLOGY, HYDERABAD, India*

Introduction: Faecal calprotectin (FC), non invasive markers of intestinal inflammation with good clinical sensitivity &useful in the differential diagnosis between inflammatory bowel disease (IBD) and irritable bowel syndrome(IBS).

Aim: Our study is to estimate FC levels in IBD and healthy controls, to correlate FC levels with clinical disease activity, to assess whether FC levels can be used to predict clinical relapse of paediatric IBD patient and to investigate if high levels were associated with a flare-up of the disease.

Method & Material: Retrospective study was done on 100 paediatric IBD: 58 ulcerative colitis(UC) & 42 crohn"s disease(CD) ,100 healthy pediatric control . Stool samples estimated FC with chemiluminescence immunoassay(CLIA)method in Dia-Sorin Liaison analyser. Baseline assessment of FC is done in all IBD for clinical disease activity cases, In follow up 37 had clinical remission for more than a year, 30 for 6-12 months and 23 for 3 to <6 months. Clinical outcome of the patients was followed up to the first relapse or up to 18 months.

Results: ***In STUDY I:*** Paediatric IBD had high FC levels (100-4000µg/g) compared with control (16-75µg/g), P < 0.0001). ***In STUDY II:*** In CD, mean FC levels 460.1±220.6 µg/g in relapsed cases during follow up,& 190.5±105 µg/g in non-relapsing cases (p=0.0039*).* ***In STUDY III*** : In UC mean ,FC levels 590±200.6 µg/g & 67±30.8 µg/g in relapsing & non-relapsing patients, (p<0.0001). In clinical relapse, 90% had FC levels ≥400 µg/g in IBD .. 82% of IBD FC levels ≤150µg/g remained in clinical remission. ***In STUDY IV*** : A FC value ≥ 150µg /g sensitivity& specificity were 97%,& 85% in predicting histological relapse, the positive predictive value of FC was 0.42 & negative predictive value was 0.75. **In *STUDY V:*** FC level ≥ 50 µg/g , the sensitivity & specificity for predicting relapse in IBD cases were 88%& 73%.In paediatric IBD when in clinical remission, If FC levels ≤150 µg/g only7.8%, risk increasing from relapse than non-relapse but with FC levels ≥150 µg/g g there was 30% risk increasing to relapse than non-relapse . A significant correlation emerged between a high FC and the probability of relapse in UC patients (P= 0.0001). Of all enrolled CD patients, FC predictability was better for ileocolonic &colonic, rather than ileal CD. only cases of colonic CD showed a significant correlation between a high FC and the probability of relapse, (P= 0.02).

Conclusions: FC proved to be stronger predictor of clinical relapse in UC than in CD, Early detection and monitoring is very important in clinical management for children, as IBD may affect their growth and sexual development.. A single moderately elevated FC is ambiguous but along with other faecal markers may enable the avoidance of invasive tests, & does provide a clear indication of when clinical intervention is required and when to change the long-term disease course.

A-404

MPB64 Secreted from Active BCG by Heating: Towards a Same-Day Diagnosis of Tuberculosis

K. NAKAISHI[1], S. Watabe[1], T. Kitagawa[1], E. Ito[2]. *[1]TAUNS Laboratories, Izunokuni, Shizuoka, Japan, [2]Waseda University, Wakamatsucho, Shinjuku, Tokyo, Japan, Japan*

Background: Tuberculosis (TB) is caused by the bacteria *Mycobacterium tuberculosis* var. tuberculosis, and it is one of the top 10 causes of death worldwide. The World Health Organization (WHO) reported that in 2017, 10 million people fell ill with TB, and 1.6 million died from the disease. Diagnosis by use of Xpert MTB/RIF (Cepheid) can be made within 2 hours, and thus it is recommended by WHO as the initial diagnostic test in all persons with signs and symptoms of TB. However, there are some critical defects in Xpert MTB/RIF: Because this method is a kind of PCR, it detects DNA not only from active tubercle bacilli but also from latent ones. If we need only to detect active tubercle bacilli in patients during treatment, Xpert MTB/RIF is not suitable. In the present study, we introduce a method to obtain active tubercle bacilli in a short time without culture. For this purpose, we heat BCG (*Mycobacterium tuberculosis* var. bovis) to stimulate BCG for secretion of MPB64, and we detect MPB64, i.e., the existence of active BCG, by an ultrasensitive ELISA assay.

Methods: We used MPB64, a specific protein secreted from active TB complex, as a biomarker. BCG was used for the TB complex. As a pre-treatment for the ultrasensitive ELISA, we warmed up BCG to enhance the secretion of MPB64 at 46°C for 1 h. In the sandwich ELISA, two specific antibodies for MPB64 were used, one of which was conjugated with alkaline phosphate (ALP). An androsterone derivative with a phosphate was hydrolyzed by ALP, and this derivative was then employed in the enzyme cycling. Consequently, MPB64 was determined by the accumulated amount of thio-NADH in the enzyme cycling.

Results: The present results showed that our ultrasensitive ELISA can detect active *Mycobacterium tuberculosis* (i.e., BCG) at the level of 300 CFU/test within only 4 hours.

Conclusion: This rapidity can contribute to the prevention of disease spread, because active TB patients can be isolated within 4 hours until the test results are obtained. The available tests for tubercle detection are a smear test, PCR and a culture test. The smear test has low sensitivity (> 10,000 CFU/mL), whereas PCR test is highly sensitive, but latent tubercle bacilli are detected in addition to active tubercle bacilli. It is very important to detect only active tubercle bacilli in hospitals because drug efficacy should be examined. The culture method can detect active tubercle bacilli, and the culture test is highly sensitive (tens CFU/mL or more) but requires a long culture period (at least 10 days and up to 2 months). Furthermore, it is possible that we apply our ultrasensitive ELISA to the sputum collected from TB patients who have been diagnosed with BD BACTEC MGIT 960 Mycobacteria Culture System. Our preliminary experiments resulted in the detection of 300CFU/test for the patient sputum. That is, our test shows the same sensitivity as the culture method. We believe that it is beneficial to detect active tubercle bacilli within 4 hours for an early diagnosis for TB.

A-405

Urbanorum or Urban Legend: A Parasite Tale

J. Monteiro, B. B. Perez, T. M. Carniato, D. R. Ramadan, M. C. Feres, S. Tufik, S. S. Andrade. *Associação Fundo de Incentivo a Pesquisa (AFIP - Medicina Diagnóstica), Sao Paulo, Brazil*

Background: Intestinal parasitosis caused by protozoa is among the major causes of disease in humans, constituting a serious public health problem, especially in developing countries with poor sanitation and lower income. An uncommon structure, first reported in 1994 in Peru, was designated *Urbanorum* spp. Since then, sporadic reports of *Urbanorum* spp. have emerged in specific Latin American countries. The current report describes the occurrence of *Urbanorum* spp. in São Paulo, the largest city of Brazil. **Methods:** From August to October 2018, the clinical parasitology section of AFIP Laboratory processed 171,131 stool specimens according to the TF-Test® protocols. This test comprises the collection of three alternate-day stool samples, simultaneously processed within the laboratory by centrifugal sedimentation, leading to parasite concentration and elimination of stool debris. Sediment suspension was transferred to a slide with one drop of Lugol´s solution, and analyzed by standard light microscopy. Only one positive stool sample per patient was considered for descriptive analysis. **Results:** During the three-month period, 171,131 stool samples were analyzed by TF-Test technique. Overall, 12,181 samples were positive for the presence of protozoa and 584 (4.8%) of them were suspected as being *Urbanorum* spp.: an yellow structure with rounded shape and hyaline features compatible as protozoa amoeboid, besides several filaments described as pseudopods. Among the 584 patients with suspected *Urbanorum* spp. detected in the study, 538 (92%) were infected with *Urbanorum* spp. only and 45 (8%) patients with two or more parasitic organisms. The prevalence of *Urbanorum* spp. infection was 67.2% and 32.4% among female and man patients, respectively. The main age range was 13 to 49 years (44.7%), while children (1-12 years of age) comprised 25% of cases. A total of 87.5% of *Urbanorum* spp. were detected in patients assisted by public health service and 12.5% assisted by the private health service. We did not have access to patient's symptoms, clinical history or further diagnostic procedures. **Conclusion:** This is the largest study to date to report the presence of *Urbanorum* spp., a suspected protozoa emerging in stool specimens in the region. In addition, our data highlight that the presence of *Urbanorum* spp. is not limited to areas of lower income and sanitation. Although further investigation is necessary to verify the organism´s true nature and its potential pathogenic role, scientific community cannot ignore the emergence and high frequency of those novel structures.

A-406

High Doses of Biotin Consumption May Interfere with Some Ortho Clinical Diagnostic VITROS 3600 Hepatitis Virus Immunoassays

M. Kodani, A. Poe, J. Drobeniuc, T. Mixson-Hayden. *Centers for Disease Control and Prevention, Atlanta, GA*

Background: Biotin taken orally can interfere with some diagnostic immunoassays, including those for thyroid hormones, ferritin, and markers of infectious disease. Assays affected are ones that use streptavidin-biotin in their design. Immunoglobulin M antibodies to Hepatitis A virus (IgM anti-HAV) is a competitive assay with potential false positives due to biotin interference based on its chemistry, while the total antibodies to Hepatitis A virus (total anti-HAV) and immunoglobulin M antibodies to Hepatitis B core antigen (IgM anti-HBc) assays, being sandwich assays, may generate false negatives on the VITROS 3600 immunodiagnostic platform. The Food and Drug Administration recommends testing up to 1200 ng/ml of biotin since this concentration can be found in individuals who consume 300 mg/day of biotin, as nutritional supplement.

Methods: The goal of our study was to examine the effect of biotin concentrations of up to 1200 ng/ml on three serology assays performed on VITROS 3600 system

- IgM anti-HAV, total anti-HAV total, and IgM anti-HBc by spiking serum samples with variable amounts of biotin. We used VITROS 3600 HBsAg, which does not use biotin, as a control. Three aliquots of positive and negative samples each were tested. The first aliquot contained 10% phosphate buffered saline (PBS), the second 300 ng/ml biotin in PBS, and the third 1200 ng/ml biotin in PBS. We tested each aliquot on the VITROS 3600 platform.

Results: Neither of the biotin concentrations affected total anti-HAV positive results (65/65 specimens) nor IgM anti-HAV negative results (59/59 specimens). However, both concentrations of biotin generated 100% false positivity (30/30 specimens) in total anti-HAV negative specimens and 8% false negativity in IgM anti-HAV positive specimens (3/39 specimens).

Conclusion: These data show that individuals taking biotin-containing nutritional supplements may test false-positive in some serologic assays using streptavidin-biotin chemistries. Further studies are warranted to determine the extent of biotin interference resulting in false positive and negative results and their impact, if any, on surveillance and diagnostic settings.

A-407

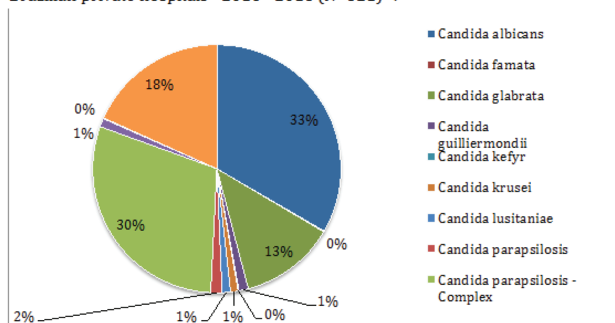
Candida Species Distribution of Blood Isolates among Patients Attending Private Hospitals

L. B. Faro, M. R. E. Aráujo, E. J. U. Kusano, L. C. Pierroti, S. E. Mezaonik, C. A. Cohn, K. Salviato, L. F. Santos. *DASA, São Paulo, Brazil*

Background: The rate of candidemia among hospitalized patients has been increased worldwide associated with several risk factors such as prolonged hospitalization, previous use of large spectrum antibiotics, surgery, venous catheter, parenteral nutrition and chemotherapy. Our goal was to evaluate the distribution of *Candida* species isolated from blood among patients attending in private hospitals in Brazil. **Methods:** We analyzed retrospectively the records from microbiology laboratory to evaluate the *Candida* species distribution among candidemia episodes occurring in patients attended at private Brazilian hospitals during 2016 to 2017. Candidemia was defined as the first isolation of any *Candida* species from at least one positive blood culture. Blood cultures were processed by automated blood culture systems (BacT/ALERT 3D® or BD BACTEC™) and microorganism identification was performed by Vitek MS® MALD-TOF mass spectrometry technology. **Results:** We identified 821 *Candida* species isolated (first isolation) from 343,468 blood cultures tests. Candidemia occurred in 766 patients, an average of 1.07 *Candida* species by patient (ranging from 1 to 3). The distribution of the *Candida* species isolated in blood cultures is illustrated in figure 1. *C. albicans* was the most frequent specie isolated (33.4%); however, *Candida* non-*albicans* represented the majority of cases (66.6%). Among 547 *Candida* non-*albicans* species, the most frequent species found were *C. parapsilosis* (244, 44.6%), *C. tropicalis* (150, 27.4%) and *C. glabrata* (102, 18.6%). When we analyzed the subgroup of non-neonates, *Candida* non-*albicans* were less prevalent (39.4%) than C. albicans (60.9%); among 33 neonates, *C. albicans* was reported in 20 cases, followed by *C. parapsilosis* - complex (10 cases, 30.3%), *C. guilhermondii* (2 cases, 6.1%), and *C. tropicalis* (1 case).

Conclusion: *Candida* non-*albicans* species were more prevalent than *C. albicans* as cause of candidemia among non-neonates patients in this study. The most frequent species isolated *C. albicans*, *C. parapsilosis*, *C. tropicalis* and *C. glabrata*.

Figure 1. Candida species distribution of blood isolates among patients attending Brazilian private hospitals - 2016 - 2018 (N=821) #.



A-408

Respiratory Viruses Detection by Real-Time Polymerase Reaction in Patients Attending Private Hospitals in Brazil

L. B. Faro, L. C. Pierrotti, A. Chebabo, J. E. Levi, C. A. Cohn, L. F. B. Porto, L. C. Scarpelli, K. Salviato, S. E. Mezaonik. *DASA, São Paulo, Brazil*

Background: Respiratory viruses (RV) are responsible for more than half of acute respiratory tract infections (RTI) and are associated with high morbidity and mortality. Rapid detection of RV using molecular biology panels can guide appropriate therapeutic management avoiding antibiotic overprescription and extra diagnostic testing. The aim of this study is to estimate the frequency of RV using a sensitive molecular detection technique in respiratory specimens from patients attending 26 private Brazilian hospitals of all ages with acute RTI.

Methods: Records from RV panel tests performed by multiplex real-time reverse transcriptase polymerase chain reaction (RT-PCR) in respiratory specimens from January 2016 to November 2017 were evaluated. The test utilized was the RT-PCR CLART® Pneumovir capable of detecting the 19 most frequent human RV causing RTI: Adenovirus (AdV), Bocavirus (BoV), Coronavirus (CoV), Enterovirus (Echovirus), Influenza A (subtypes H1N1 and H3N2), Influenza B, Influenza C, Metapneumovirus A and B, Parainfluenza 1, 2, 3 and 4, Rhinovirus, and Respiratory Syncytial Virus (RSV) A and B. Virus detection is performed by RT-PCR amplification of a specific 120-330 bp fragment of the viral genome.

Results: A total of 7,168 tests were performed for 6,447 patients; 51% of them were male. The average test per patient was 1.1. A total of 5,440 pathogens were detected. The distribution of pathogens is demonstrated in table 1. The most frequent pathogens detected were RSV (30.5%) Rhinovirus (18.1%) and Influenza A(H1N1) (11.1%). Unusual pathogens were also detected - BoV (8.1%), CoV (0.1%); Influenza C virus was detected in 44 samples.

Conclusion: Rhinovirus and RSV were the most prevalent virus detected in respiratory specimens from patients with RTI in Brazil. The use of multiplex RT-PCR was able to detect a large number of RV including the unusual and mostly recently described RV, including Influenza C, CoV and Bocavirus.

Table 1. Respiratory virus distribution in respiratory samples among patients attending private hospitals in Brazil detected by multiplex RT-PCR – Jan 2016 to December 2017 (N=5,440 pathogens).

Respiratory viruses	N	%
Adenovirus	382	7,0%
Bocavirus	440	8,1%
Coronavirus	6	0,1%
Enterovirus (Echovirus)	232	4,3%
Influenza A virus	85	1,6%
Influenza A H3N2	113	2,1%
Influenza A virus H1N1 /2009	606	11,1%
Influenza B virus	181	3,3%
Influenza C virus	44	0,8%
Metapneumovirus	246	4,5%
Parainfluenza virus 1	31	0,6%
Parainfluenza virus 2	25	0,5%
Parainfluenza virus 3	299	5,5%
Parainfluenza virus 4	110	2,0%
Rhinovirus	982	18,1%
Respiratory Syncytial Virus A	969	17,8%
Respiratory Syncytial Virus B	689	12,7%
TOTAL	5440	100,0%

A-409

Testing Anti-Zika Virus NS1 IgA Additionally to IgM Increases Sensitivity in Acutely Infected Patients from Regions Endemic for Flaviviruses

K. Steinhagen[1], N. Muigg[1], O. Sendscheid[2], W. Schlumberger[1]. [1]*Institute for Experimental Immunology, affiliated to EUROIMMUN, Luebeck, Germany*, [2]*EUROIMMUN US, Inc., Mountain Lakes, NJ*

Background: Specific IgM response to Zika virus (ZIKV) can be low or absent in patients with acute ZIKV infection and a history of other infections with related flaviviruses, e. g. dengue virus (DENV), presenting with an early high IgG titer. In these ZIKV cases, IgA against ZIKV non-structural protein 1 (NS1) was observed in the acute phase, suggesting anti-ZIKV IgA as alternative acute marker in secondary infec-

tions. In this study, we investigated the diagnostic benefit of an ELISA for combined detection of anti-ZIKV NS1 IgA and IgM.

Methods: The following human serum panels were included in this study: [1] A sensitivity panel (panel 1) comprising acute serum samples (day 8-16 post symptom onset) of 31 residents from the Dominican Republic (2015), where ZIKV and DENV are endemic. Patients had been tested positive for ZIKV RNA and anti-DENV IgG during the viremic phase (≤ day 5). [2] A specificity panel (panel 2) consisting of serum samples (day 3-7 post symptom onset) of 40 Vietnamese patients, hospitalized with DENV hemorrhagic fever according to the World Health Organization case definition grade I and tested positive for DENV nucleic acid and anti-DENV IgG. Vietnam (2015) is endemic for DENV but not for ZIKV.Anti-ZIKV NS1 antibodies were determined in each sample using a commercial NS1-based Anti-Zika virus ELISA IgM (Euroimmun AG, Germany) and a corresponding ELISA (Euroimmun), applying a combination of anti-human IgA/IgM conjugated with peroxidase.

Results: In panel 1, 29% (9/31) of samples were positive for anti-ZIKV NS1 IgM, whereas 100% were positive for combined specific IgA and IgM. In panel 2, none of the sera reacted in the Anti-Zika virus ELISA IgM, two samples were reactive in the Anti-Zika virus IgAM ELISA (5.0%).

Conclusion: As patients with acute ZIKV infection from flavivirus endemic regions may not develop NS1-specific antibodies class IgM, additional testing of anti-ZIKV NS1 IgA is required.

A-410

Yellow Fever Outbreak in Brazil 2018: Laboratory Experience

L. T. Galindo, A. D. Hristov, L. Scarpelli, L. C. Pierroti, A. Chebabo, J. E. Levi. *DASA Laboratories, Barueri, Brazil*

Background: In 2017-2018 Brazil has experienced the largest outbreak of Yellow Fever recorded in the last 40 years, with more than 2,000 confirmed cases and 400 deaths. Differently from past outbreaks, the current one spread to more populated areas in the Southeast region, raising the fear of urbanization, which has not occurred so far. This risk led to huge immunization campaigns, in about 6 months, millions were vaccinated with the 17D attenuated strain. Accurate diagnostics of suspicious cases and vaccine adverse reactions became urgent.

Methods: Patients seeking for laboratory diagnosis of YFV had a blood sample collected in one of the patient services centers belonging to the DASA group. According to the medical prescription, samples were submitted to IgG and/or IgM testing by an indirect immunofluorescence commercial assay. Direct detection of viral RNA in plasma was performed by use of a real-time PCR assay developed by DASA. This assay is able to discriminate the wild-type virus from the vaccine 17DD strain.

Results: In between January and March 2018, 394 samples were submitted to IgG testing and 184 (47%) were reactive, while 382 IgM tests were performed and 41(11%) found reactive. Eighty-nine patients were tested for the presence of viral RNA being 17 reactive; 9 were shown to harbor the wild-type circulating strain while 8 presented a positive result for the vaccine strain.

Conclusion: In general, PCR was requested for symptomatic patients, the majority hospitalized when the distinction between the YFV strains was important both for prognostic and epidemiological reasons. IgM was more frequently used, probably due to the wider knowledge of this marker, but also to the longer diagnostic window. IgG was the most used test, probably in part to check for immunity of previously vaccinated subjects, what is contraindicated. Having validated diagnostic tools available was key to assist the country in managing this deadly epidemic.

A-411

Comparison of Enzyme-Linked Immunosorbent Assays for the Diagnosis of Dengue Virus Infections

A. Gebhardt[1], O. Klemens[1], O. Sendscheid[2], K. Stiba[1], S. Saschenbrecker[1], K. Steinhagen[1]. *[1]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany, [2]EUROIMMUN US, Inc., Mountain Lakes, NJ*

Background: Dengue virus (DENV) is a mosquito-borne flavivirus that causes a febrile illness in humans, occasionally leading to severe hemorrhagic fever and shock syndrome. After viraemia, the laboratory diagnosis is based on antibody detection which can be complicated by cross-reactivity of the antibodies with other flaviviruses, mainly due to antigenic substrates containing the highly conserved viral glycoprotein E. Flavivirus serological assays based on the more species-specific non-structural protein 1 (NS1) have demonstrated a higher specificity. Here, we compared the performance of two ELISAs based on different DENV antigens.

Methods: The study included 124 follow-up samples (day 0-196) from DENV-infected travelers. Additionally, we examined sera from 688 healthy individuals and 95 patients with previous flavivirus contact, i.e. vaccination against or infection with tick-borne encephalitis virus (TBEV), yellow fever virus (YFV), hepatitis C virus (HCV), West Nile virus (WNV) or Zika virus (ZIKV). Samples were analyzed for anti-DENV IgM and IgG using the Anti-Dengue Virus Type 1-4 ELISA (Euroimmun AG, Lübeck, Germany) based on purified DENV particles and recombinant glycoproteins E of DENV1-4. In addition, IgG reactivity against DENV NS1 was examined by an ELISA (Euroimmun) coated with recombinant NS1 of DENV1-4

Results: Of the samples taken on day 0, anti-DENV1-4 antibodies were positive in 36% (IgM) and 40% (IgG), but only 13% reacted positively in the NS1-based IgG ELISA. Among sera obtained between day 6 and 14, 100% were anti-DENV1-4 positive for both IgM and IgG, while anti-DENV NS1 IgG positivity was detected in 70% of cases. In samples taken later, the prevalence of anti-DENV1-4 IgM steadily decreased, whereas both IgG ELISAs reached positivity in 100%. Specificities of the different ELISAs were 94-99% with respect to healthy individuals. However, in the cross-reactivity panel, the NS1-based IgG ELISA was more specific than the anti-DENV1-4 IgG ELISA (83% vs. 49%), with deviations observed in TBEV-vaccinated (100% vs. 68%) and WNV-infected (93% vs. 33%) patients. Highest cross-reactivity (100%) occurred with anti-ZIKV IgG antibodies.

Conclusion: The Anti-Dengue Virus Type 1-4 ELISA IgM and IgG is capable of detecting all DENV infections in the time window predestined for serodiagnostics, thus presenting a valuable screening tool for acute infections and epidemiological studies. By comparison, the NS1-based IgG ELISA has the advantage of reduced IgG cross-reactivity, but limited sensitivity in early stages of infection.

A-412

Novel Screening ELISA for Sensitive Detection of Mayaro Virus-infected Patients

M. Moraes Soane[1], D. Freitas Henriques[2], J. Abreu Lima[2], L. Caricio Martins[2], N. Muigg[3], O. Sendscheid[4], O. Klemens[3], K. Steinhagen[3]. *[1]Euroimmun Brasil, São Paulo, Brazil, [2]Seção de Arbovirologia e Febres Hemorrágicas, Instituto Evandro Chagas, Ananindeua, Brazil, [3]Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany, [4]EUROIMMUN US, Inc., Mountain Lakes, NJ*

Background: Mayaro virus (MAYV) is an emerging alphavirus circulating in the Caribbean and South America. It is transmitted to humans via mosquito bites, causing a febrile illness, often with prolonged arthralgia, which resembles other infections with co-circulating arboviruses, e. g. dengue virus (DENV), chikungunya virus (CHIKV) or Oropouche virus (OROV). Serological differentiation of MAYV from other alphavirus infections is complicated in the presence of antibodies targeting homologous antigens from related viruses (Semliki Forest virus complex), primarily CHIKV. Still, antibody detection can extend the time for diagnosing acute MAYV infections beyond the short viraemic period suitable for MAYV RNA detection. Here, serum samples of patients with MAYV or other arboviral infections were analysed with a novel Anti-Mayaro Virus ELISA (Euroimmun AG, Germany) for the detection of specific IgM and IgG at the Instituto Evandro Chagas (Ananindeua, Brazil).

Methods: The serum samples originated from Brazilian patients with clinically and serologically characterised febrile infections, drawn between day 11 and 117 post symptom onset. Pre-characterisation included analyses for IgM and haemagglutination inhibition (HI) antibodies against MAYV, CHIKV, DENV, yellow fever virus (YFV), Zika virus (ZIKV), OROV, eastern and western equine encephalomyelitis virus (EEEV and WEEV) and flaviviruses in general using the respective in-house IgM antibody capture (MAC) ELISAs and indirect haemagglutination inhibition assays. The first panel encompassed 46 samples, including 21 positive and 25 negative for anti-MAYV IgM and HI antibodies. The second panel (n = 12) consisted of six anti-MAYV HI antibody-positive and six anti-MAYV HI antibody-negative samples. Samples were investigated using the Anti-Mayaro Virus ELISA (Euroimmun) for the detection of IgM (panel 1) and IgG (panel 2).

Results: In panel 1, the Anti-Mayaro Virus ELISA IgM was 100% (21/21) sensitive and 76% (19/25) specific. The six discrepant samples had been pre-characterised as positive for CHIKV (5/11) or general flavivirus (1/3) infection. Analysing panel 2, the Anti-Mayaro Virus ELISA IgG revealed a sensitivity of 100% (6/6), at 50% (3/6) specificity. Three samples with anti-CHIKV HI antibody-positive but anti-MAYV HI antibody-negative pre-characterisation were positive in the ELISA.

Conclusion: The novel Anti-Mayaro Virus ELISAs (IgM and IgG) showed a high sensitivity at moderate specificity. This specificity meets the expectations and, in the

majority of cases, can be explained by cross-reactivity with antibodies against related viruses, primarily CHIKV. Thus, the ELISAs are suitable as screening assays, reliably detecting MAYV-infected patients.

A-413

Influenza A, Influenza B, and RSV Multiplex, Whole Process Control Made Using Recombinant Virus Technology

J. Leach[1], E. Morreale[1], C. Huang[2], L. Nguyen[2], B. Anekella[2]. [1]*SeraCare Life Sciences, Milford, MA,* [2]*SeraCare Life Sciences, Gaithersburg, MD*

Background: Reverse Transcription-Polymerase Chain Reaction (RT-PCR), and other nucleic acid amplification tests can detect influenza and Respiratory syncytial virus (RSV) RNA in respiratory specimens with high sensitivity and high specificity. These tests can provide results in as little as 30 minutes and CDC guidelines recommend their use for all hospitalized patients with flu-like symptoms. Clinical labs that are validating new tests or performing routine QC of their respiratory virus tests need stable, reproducibly manufactured, low positive reference materials for influenza and RSV. SeraCare has used AccuPlex® recombinant virus technology to produce a multiplexed reference material that mimics clinical samples because it contains the complete genomic RNA of the pathogen, goes through the entire extraction procedure and is non-infectious to ensure safety for lab personnel.

Methods: SeraCare developed the AccuPlex Recombinant Multiplex Flu and RSV reference material to meet this need. The entire genomic RNA sequences from influenza A(H1N1)pdm09 lineage, Influenza B/Victoria lineage, and RSV were used in the design of the recombinant viruses. The recombinant viruses are replication deficient and are also heat treated to further ensure the product is non-infectious. The recombinant viral stocks were diluted in a viral transport media, and the concentration of each preparation was verified by droplet digital PCR. The reference material was tested functionally using Cepheid Xpert® Xpress Flu/RSV as well as BioFire® FilmArray® Respiratory Panel.

Results: The multiplex Flu and RSV recombinant viruses are designed to be used as full process control. These reference material yields POSITIVE test results for influenza A (Flu A), influenza B (Flu B), and RSV viral RNA on the Cepheid GeneXpert system. The reference material yields POSITIVE test results for influenza A-H1-2009, influenza B and RSV on the BioFire FilmArray RP2 panel. The multiplexed nature of the reference material allowed routine QC of primers/probes specific for FluA, FluB and RSV in a single test, which preserves more of the test kit for unknown samples. Real time stability studies for similar AccuPlex recombinant virus reference materials show no changes in titer by real time PCR though 5 years of storage at either - 20 ºC or +4 ºC.

Conclusion: SeraCare has developed a stable, non-infectious, multiplexed, whole process reference material for FluA, FluB and RSV. The material can be used as part of a laboratory quality control program for routine monitoring of test performance, as well as validation, competency evaluation and lot release testing. The material also demonstrates how AccuPlex recombinant virus technology can be used to make multiplexed reference materials that contain the entire genomic RNA content of selected pathogens.

A-414

Dried Blood Spots Represent Well-suited Specimens for Detection of Anti-Trypanosoma Cruzi Antibodies

A. C. Franke[1], F. Lindhorst[2], O. Sendscheid[3], K. Steinhagen[2]. [1]*University of Lübeck, Luebeck, Germany,* [2]*Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Luebeck, Germany,* [3]*EUROIMMUN US, Inc., Mountain Lakes, NJ*

Background: The parasitic kinetoplast Trypanosoma cruzi can be found in warm rural areas. In Brazil, the estimated seroprevalence of T. cruzi is 1-6%. CDC estimates that more than 300,000 persons with Trypanosoma cruzi infection live in the United States. Transmission occurs by hematophagous bugs and infection causes Chagas disease, which can be fatal if untreated. Diagnosis is usually performed by microscopic detection in blood smears (early during infection), by xenodiagnostics or by detection of specific antibodies. Blood spotting onto filter paper is an easy and well-established method for collecting specimens used in serology. After drying, analytes such as antibodies can be extracted from these Dried Blood Spots (DBS) and subsequently be used for the diagnosis of different diseases. For efficient analysis by ELISA, DBS have to meet several requirements, including comparability to serum (gold standard), reproducibility of extraction, stability during storage and transport. In this study, we analyzed the extraction of anti-T. cruzi IgG from DBS and the detection of these antibodies using the Anti-Trypanosoma cruzi IgG ELISA (Euroimmun AG, Germany).

Methods: To validate DBS as proper specimens, 12 paired samples covering a wide range of the calibration curve were analyzed in triplicate. As DBS lose reactivity over time, the extraction volume was adapted such that freshly produced DBS show 120% recovery compared to the respective serum (diluted 1:101). Reproducibility was examined in 10 measurements at 5 days including sample duplicates. DBS were stored in a closed bag with desiccant at -20°C, 4°C, RT or 37°C for 4 weeks. Measurements were performed after 1, 2 and 4 weeks. To test for stability during transport, DBS were stored at 30°C in a closed bag with desiccant or in an open bag for 3 weeks, and measured weekly. In addition, we examined DBS obtained from 440 pregnant women from Brazil.

Results: The detection of anti-T. cruzi IgG from DBS showed high reproducibility as well as 100% sensitivity and specificity compared to serum analysis. Storage of DBS over 4 weeks at -20°C, 4°C or RT resulted in a temperature-independent loss of reactivity of 20% on average. DBS stored at 37°C showed 78% recovery compared to serum after 4 weeks. Among 440 DBS from pregnant Brazilian women, 5 positive or equivocal results could be confirmed in duplicates resulting in a seroprevalence of 1.1%.

Conclusion: DBS fulfill all conditions for efficient serological diagnostics. Due to their high-temperature stability and simplicity of collection, DBS can be used in rural areas with less infrastructure or in serological studies: blood is collected on filter paper by the patient and then sent to a laboratory for analysis without the need for permanent cooling. It has been shown that also other immunoglobulin isotypes can be extracted and subsequently detected from DBS. Automated punching systems for DBS facilitate the entire process.

A-415

Urinary Tract Infections: Frequency of Microorganisms and Antimicrobial Profile from Brazilian Public Hospitals

J. Monteiro, F. M. Inoue, A. T. Lobo, D. R. Ramadan, M. D´Martino, S. S. Andrade, S. Tufik. *Associação Fundo de Incentivo a Pesquisa (AFIP - Medicina Diagnóstica), São Paulo, Brazil*

Background: Urinary tract infections (UTIs) are one of the most common bacterial infections in worldwide and represent a worried public health problem, especially due to a significant rise in global antibiotic use in recent years. The purpose of the study was described the main pathogens found in urine samples and the antimicrobial resistance profiles among inpatients at public hospitals of the biggest city of Brazil. **Methods:** We analyzed 72,096 urinary samples isolated from 12 public hospitals of São Paulo city during July 2017 to July 2018. The clinical samples were cultivated onto ChromID® CPS agar plates and then incubated at 35°C for 18-24 h. The bacteria identification was performed by MALDI-TOF mass spectrometry (Vitek-MS) and the minimal inhibitory concentrations of antibiotics were determined using the Vitek®2 system. Only positive results with one bacterial species and a colony count ≥ 10^5 CFU/mL was considered for descriptive analysis. **Results:** Of the 72,096 urine samples tested, 12,798 (17.7%) samples were positive for the presence of microorganisms. Out of which, 76.9% were Gram-negative Bacilli (GNB), 14.2% were Gram-positive Cocci (GPC) and 8.9% were yeasts. The order of prevalence (top ten) of uropathogens isolates was *Escherichia coli* [6053/12798 (47.3%)], *Klebsiella* spp. [1766/12798 (13.8%)], *Proteus* spp. [703/12798 (5.5%)], *Enterococcus faecalis* [658/12798 (5.1%)], *Candida albicans* [574/12798 (4.5%)], *Candida* non-albicans [558/12798 (4.4%)], *Streptococcus* β-hemolytic [566/12798 (4.4%)], *Pseudomonas aeruginosa* [437/12.798 (3.4%)], Coagulase Negative *Staphylococcus* (CNS) [340/12.798 (2.7%)] and *Enterobacter* spp. [331/12798 (2.6%)]. Extended spectrum beta-lactamase (ESβL) rates for *E. coli* and *K. pneumoniae* were 15.2% (921/6053) and 62.1% (1097/1766), respectively. The resistance to carbapenens was observed mainly in 88.9% *Acinetobacter spp.* (96/108), 32.6% *K. pneumoniae* (575/1766), 30.2% *Pseudomonas aeruginosa* (132/437), 12.1% *Enterobacter* spp. (40/331) and 0.27% (20/7191) of others GNB. Between the GPC the resistance to vancomycin was observed mainly in 58.3% *E. faecium* (49/84) and 10.8% *E. faecalis* (71/658) and the resistance to oxacillin was observed in 42.2% *S. aureus* (68/161) and 27.9% of CNS (95/340). **Conclusions:** This data could be used locally with other related studies, to properly interpret significant resistance patterns and choose the most appropriate antimicrobial for empirical treatment of inpatients with severe urinary tract infections.

A-416

Emergence and Clonal Diversity of New Delhi Metallo Beta-Lactamase Producing *Enterobacteriaceae* in Brazil

J. Monteiro, F. M. Inoue, A. T. Lobo, D. R. Ramadan, M. C. Feres, S. Tufik, S. S. Andrade. *Associação Fundo de Incentivo a Pesquisa (AFIP - Medicina Diagnóstica), São Paulo, Brazil*

Background: Infections caused by carbapenem-resistant *Enterobactereaceae* are of great clinical and epidemiological importance due to their rapid dissemination and high mortality rates. In this scenario, New Delhi Metallo β-Lactamase (NDM) has become one of the main globally described carbapenemases. The aim of this study was report the detection of NDM-1 producing *Enterobacteriaceae* clinical isolates recovered from Brazilian hospitals. **Methods:** From January 2015 to January 2019, the clinical microbiology section of AFIP Laboratory detected 65 carbapenem-resistant Enterobacteriaceae isolates with positive screening assay of Metallo-β-lactamase producer, using commercially available disks containing ertapenem, imipenem and meropenem with and without EDTA (0.1 M). These strains were isolated from blood (n=7), wound (n=5), bone fragment (n= 3), fluid culture (n=3), catheter tip (n=4), urine (n=24) and rectal swab (n=19) from patients hospitalized in twenty-three tertiary Brazilian hospitals. The bacterial identification was performed by Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS) and the minimal inhibitory concentration of antibiotics was determined using the Vitek 2 System. The detection of the carbapenemase (bla_{KPC}, bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{GES} and $bla_{OXA\text{-}48\text{-}like}$), ESβL ($bla_{TEM}$, bla_{SHV} and $bla_{CTX\text{-}M}$) and *qnr* genes were determined by real time PCR. Genetic relatedness of the strains was characterized by Pulsed-Field Gel Electrophoresis (PFGE) after DNA digestion with *Spe*I. **Results:** Among the 65 Enterobacteriaceae studied, 49 isolates were identified as *Klebsiella pneumoniae* (KPN), ten as *Providencia rettgeri*, two as *Providencia stuartii,* three as *Proteus mirabilis* and one as *Citrobacter freundii*. In all of them, high-level of resistance were detected to ceftazidime, ceftriaxone, cefepime, ertapenem, imipenem and meropenem. Between KPN isolates, 67.3%, 12.2%, 30.6% and 6.1% were resistant to gentamicin, amikacin, tigecycline and colistin, respectively. All 65 *Enterobacteriaceae* isolates showed screening as possible MβL producers. All of them harbored bla_{NDM} gene and one KPN also harbored bla_{KPN} gene. The presence of bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M}$, *qnr*S and *qnr*B genes were detected in 83%, 75.4%, 83%, 32.3% and 15.4% of the strains tested, respectively. PFGE was performed in 35/49 KPN and the analysis showed 23 different patterns (A to X), of which eight patterns were identified in more than one strain, at the same hospital. Out of ten *P. rettgeri* strains, the PFGE was performed in seven and showed three patterns denominated as PA (five strains), PB and PC (one strain, each). **Conclusion:** All the strains harboured bla_{NDM} associated to one or more ESβLs and *qnr* genes and were considered multidrug resistant. The findings of the several PFGE patterns suggest the occurrence of dissemination of bla_{NDM}-harboring plasmid in Gram-negative bacilli isolated from Brazilian hospitals. An early detection of the mechanism of resistance by screening methods could be very helpful for clinical outcome, surveillance and infection control measures.

A-417

GeneXpert MTB RIF Assay Performance in Detecting *Mycobacterium Tuberculosis* and Rifampicin Resistance among Patients Attended at Private Hospitals in Brazil

L. B. Faro, L. C. Pierrotti, C. A. Cohn, A. Chebabo, L. F. Santos, K. Salviato, L. Rodrigues, N. Maluf, L. F. B. Porto. *DASA, São Paulo, Brazil*

Background: *Mycobacteria tuberculosis* (MTb) remains a major global health problem causing millions of new tuberculosis (TB) cases each year with unacceptably high mortality. Timely diagnosis of TB is extremely important to optimize the treatment and cure rates. GeneXpert MTB/RIF (Xpert) is a real-time PCR test able to detect *M. tuberculosis* DNA in clinical specimens and rifampicin (RFM) resistance based on *rpo*B mutations. **Objective:** To report the GeneXpert MT/RIF results used as a rapid point-of-care diagnosis in a private hospital in Brazil, from 2014 to 2018. **Methods:** We reviewed retrospectively all GeneXpert MTB/RIF results performed in patients with clinical suspicion of TB attended at private hospitals in Brazil from 2014 to 2018. All tests were performed at the DASA laboratory. **Results:** Throughout the study period, a total of 1,978 samples were tested; 135 duplicate tests were excluded resulting in 1,843 analyzed results in a total of 1,843 patients. The median age was 45 years and 55.4% was male. Respiratory samples represented the majority of clinical specimens (65.8%). DNA M. tuberculosis detection was reported in 112 samples (6.1%). Only two samples were positive for RFM resistance. Table 1 shows the GeneXpert MTB/RIF (Xpert) results according clinical specimens. **Conclusion:** GeneXpert MTB/RIF assay was helpful for TB diagnosis in respiratory and non-reparatory samples. RFM resistance detected by *rbo*B mutations was 1.8%, which was considered similar compared to national rates of MTb resistance ranging from 2 to 4%. Rifampicin resistance can be used as a surrogate marker for multidrug resistance TB.

Table 1. GeneXpert MTB/RIF (Xpert) results according clinical specimens in patients attending private hospitals in Brazil – 2014 – 2018.

Clinical Specimen	Total	Positive		RFM resistance	
	N	N	%	N	%
Respiratory samples	1213	96	7.9%	1 #	1.0%
Tissues 1	51	4	7.8%		
Urine	43	2	4.7%		
Cavity effusions 2	153	7	4.6%	1 ##	0.7%
CSF	132	0	0		
Blood marrow and peripheral blood	204	2	1.0%		
Secretions	40	1	2.5%		
Unknown	7	0	0		
TOTAL	1,843	112	6.1%	2	1.8%

CSF, cerebrospinal fluid;
1. Lung biopsy sample (1 case); 2. Pleural effusion samples (144 cases); #. Sputum sample; ## Pleural effusion sample.

A-418

Changes of Early Damage Indexs of Renal Function in Patients with Viral Hepatitis and Cirrhosis

X. Weiyi, L. Junxia, Y. Qian, X. Qing. *The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: To understand the renal function injury of patients with viral hepatitis and cirrhosis, and to screen the effective indicators that can early indicate the renal function injury.

Methods: 61 patients with viral hepatitis and 63 patients with cirrhosis from the First Affiliated Hospital of Zhejiang University School of Medicine were enrolled as the viral hepatitis group and the cirrhosis group, and their blood and urine samples were collected. In addition, urine specimens of 60 healthy subjects were collected as the control group, and indicators such as Creatinine, Urea Nitrogen, Urine Protein(UP), β2-microglobulin(β_2-MG), Microalbumin(mAlb), Immunoglobulin G(IgG) and Retinol-Binding Protein(RBP) were detected in urine specimens of each group. Serum liver biochemical parameters were also detected in patients with viral hepatitis and cirrhosis. Statistical analysis was performed on the changes of early renal function markers in the viral hepatitis group, cirrhosis group and healthy control group, and the indicators sensitive to early renal damage were screened.

Results: The levels of UP, Uβ_2-MG, URBP, UIgG and UmAlb in early renal function injury indicators of the viral hepatitis group and the cirrhosis group were all higher than those of the control group, with significant differences ($P<0.01$). The serum TP and Alb levels in the cirrhosis group were lower than those in the viral hepatitis group, and UP/Cr and CYC levels were higher than those in the viral hepatitis group, with statistically significant differences ($P<0.05$), among which serum CYC, TP, Alb and UREA were significantly different ($P<0.01$). There was no significant difference in other indexes of early renal function injury ($P>0.05$).

Conclusion: Changes in urine levels of β2-MG, mAlb, IgG, and RBP may suggest early renal injury in patients with cirrhosis and viral hepatitis, and timely guide clinical diagnosis and treatment.

A-419

Clinical Validation of Quantiferon-Citomegalovirus and Quantiferon-Monitor Testing for Immunity Cell Evaluation in Kidney Transplant Recipients and Healthy Individuals

L. C. Pierrotti[1], V. V. Frade[1], P. Udiloff[1], C. M. Dias[1], C. Khawali[1], E. D. Netto[2], G. M. Melo[1], L. Rodrigues[1]. [1]*DASA, São Paulo, Brazil,* [2]*USP, São Paulo, Brazil*

Background: Quantiferon (QF)-Monitor and QF-Cytomegalovirus (CMV) are in vitro diagnostic tests designed to evaluate cell-mediated immune (CMI) function by measuring interferon-gamma (IFN-γ) in plasma by enzyme-linked immunosorbent assay (ELISA). QF-Monitor assay predicts intensity of immunosuppression state and consequent risk of rejection and infections. QF-CMV predicts risk of CMV replication and disease. Those tests have no comparative method to allow analytical validation. The aim of this study was to perform clinical validation based on the premise of their ability to measure the CMI among kidney transplant recipients (KTR) and healthy

individuals (control group) in different clinical scenarios. **Methods:** QF-Monitor and QF-CMV were performed according to manufacture's instructions (QIAGEN). Briefly, in the QF-Monitor assay peripheral blood mononuclear cells are stimulated with QFMLyoSphere to assess the innate and adaptive immune system. QF-Monitor results are expressed in high, moderate or low IFN-γ production. QF-CMV assay are based on three blood tubes: 1) stimulated with CMV specific antigens; 2) unstimulated used as a negative control; 3) stimulated with mitogen used as positive control. Final results are expressed as IFN-γ production calculated by the measured value in the CMV-tube minus the measured value in the negative control and are classified as negative, positive or indeterminate results. KTR were recruited from a public university hospital and health controls were recruited among laboratory professionals. The study was approved by Ethical Committee at the university hospital. **Results:** Table 1 summarized the data. A total of 47 KTR and 34 healthy individuals control were categorized according the pre-test probability results based on clinical scenario. **Conclusions:** Based on the clinical validation process, QF-CMV test presented 100% of specificity and 78% of specificity among KTR and 100% of specificity and 88% of sensitivity among the healthy individuals. QF-Monitor presented 100% of specificity validated among KTR and 100% sensitivity validated among healthy individuals.

Table 1. Quantiferon-CMV results among KTR and healthy individuals controls according to the expected pre-test resuts groups:

Group	Criteria	Pre-test Expected results	QF-CMV results
Kidney Transplant Recipients			
A1	Time since transplantation ≤ 30 days or Total lymphocytes < 200 cells/mm^3 OR CMV-seronegative	QF-CMV: negative	Number of patients = 3 Results: 3 negative **100% specificity**
A2	Time since transplantation > 30 days and < 12 months or Total lymphocytes > 200 cells/mm^3	QF-CMV: negative/ or positive	Number of patients =17 Results: 1 (6%) indeterminate 7 (41%) negative 9 (53%) positive
A3	Time since transplantation ≥ 12 months or Total lymphocytes > 800 cells/mm^3 No rejection treatment during the last 6 months	QF-CMV: positive	Number of patients = 27 Results: 2 (7%) indeterminate 4 (15%) negative 21 (78%) positive **- 78% specificity**
Healthy Individuals			
B1	CMV-seronegative	QF-CMV: negative	Number of patients = 2 Result: 2 negative **- 100% specificity**
B2	CMV-seropositive	QF-CMV: positive	Number of patients = 32 Results: 28 (88%) positive **- 88% sensitivity**

A-420

H1N1: A Retrospective Study from Brazilian Landscape between 2016 and 2018

F. L. O. Marinho, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: The influenza A (H1N1) virus causes seasonal epidemics that result in severe illnesses and deaths almost every year. This virus has high transmissibility, a short incubation period, and high rates of morbidity and mortality. According to the World Health Organization (WHO), the H1N1 pdm09 has affected more than 214 countries and caused more than 18,449 deaths. After the pandemic, influenza A (H1N1) pdm09 replaced the previously circulating seasonal H1N1 and has since remained in seasonal circulation. In Brazil, 44,544 cases of the disease and 2051 deaths were reported from July 2009 to January, 2010. However, the total number of cases and deaths were likely much higher than the notified number. Given the mortality rate during the 2009/2010 influenza A(H1N1)pdm09 pandemic and the continued seasonal circulation of this virus, vigilance must be maintained and emphasizes the need for a more accurate epidemiological survey, especially in developing countries. **Objective**: To describe the prevalence rate of H1N1 virus infection in Brazil and federative units during period of 2016 to 2018.

Methods: This was a retrospective study, carried out through consultation of laboratory test results stored in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) web LIS. All results of a H1N1 obtained and released from January 2016 to December 2018 were compiled. Epidemiological data such as gender, age and region of the country of H1N1 patients were statistically analyzed by SPSS v.19

Results: A total of 1255 patients from all over the country were evaluated between 2016 and 2018, thus 47.3% were male and 52.7% were female. There was a predominance of patients from the Southeast region (62.1%), followed by the Midwest (15.9%), Northeast (13.6%), South (7.8%), and North (0.6%). The rate of positivity for H1N1 was 18.8% for the whole period evaluated. Of this total, 53.8% were female and 46.2% were male, and the prevalence per year was 21.4% in 2016, 13.4% in 2017 and 21.6% in 2018. About age group, the distribution was 4.2% up to one year, 16.9% between 2 to 10 years, 7.6% 11 to 20 years, 8.1% 21 to 30 years, 16.5% 31 to 40 years, 11.9% 41 to 50 years, 12.3% 51 to 60 years, 9.7% 61 to 70 years and 12.7% were over 70 years old.

Conclusion: The number of tests has been increasing in the last three years, the highest rate of positivity were in children (2 to 10 years old) followed by the age group 31 to 40 years. Last two years (2017 and 2018) women were more infected than men, while in 2016, it was the opposite. The current study provides the overall influenza prevalence rate and information about circulating of H1N1virus in different geographical areas of Brazil. Epidemiological studies can be used for several purposes such as identify the dominant types of the virus to vaccination strategies and identify pattern of the disease.

A-421

Saint Louis Encephalitis Virus (SLEV): Analytical Validation of Molecular Method for Rapid Detection for Control Measures and Epidemiological Studies

J. P. Silva, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Saint Louis Encephalitis Virus (SLEV) is arthropod-borne virus (family *Flaviviridae*, genus *Flavivirus*), which has its RNA genome of approximately 11,000 kb. This arbovirus circulates in natural transmission cycles involving primarily *Culex* species mosquitoes and birds; humans and other vertebrates are thought to be incidental hosts. Clinical disease can vary from mild symptoms, including febrile illness and headache, to severe disease, including meningitis and encephalitis. Since its discovery in 1933, the SLEV widely dispersed in North and South America and is considered endemic on this continent, with cases being diagnosed from Canada to Argentina. This fact indicates that there is an urgent need to increase the current knowledge about this virus in order to diagnostic and prevent future cases. In the diagnostic laboratory, the molecular assays such as real-time PCR for the detection of SLEV hold greater promise for the detection of this virus in human specimens.

Objective: To describe the analytical validation of one step real-time PCR assay for detection of Saint Louis Encephalitis Virus.

Methods: The primer-probe sets were designed from two conserved regions of the SLEV genome (GenBank accession no. M16614): envelope glycoprotein/non-structural NS1 protein (ENV/NS1) and membrane M protein (MMP), respectively. One-Step reagents were used to directly amplify RNA samples on real-time PCR instrument. Performance of assay was evaluated using commercial quantified positive control and the parameters of analyze included: (i) In silico analysis of primer-probe sets by bioinformatics tools; (ii) Determination of threshold (Dilution of RNA which were run in triplicate); (iii) Analytical sensitivity (Limit of detection with concentration of RNA in the range of 1.000 to 31 copies/μL); (iv) Intrassay and interassay precision; (v) Analytical specificity (Interference study with Dengue virus, Chikungunya virus, Zika virus, Yellow fever virus and West Nile Virus); (vi) Test of spike in.

Results: The sequences of the primer-probe sets were compared to an alignment of 23 SLE virus structural region sequences, and two primer pairs that demonstrated maximum homology to all SLE virus strains were selected. The detection limits of test were 62 copies/μL for ENV/NS1 target and 125 copies/μL for MMP target with 95% confidence interval. The regression equations obtained show good amplification conditions with positive correlation between the variables, with a coefficient of determination (r^2) of 0.99. The assay demonstrated no cross-reactions with closely related viruses. The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility. The spiked sample presented positive results with a minimum value of 3.750 copies/mL of sample.

Conclusion and Perspectives: Real-time PCR can play an important role in the rapid and sensitive diagnosis of arbovirus infections, as long as the protocols are standardized locally in all involved steps. This analytical validation provided data indicating a high specificity and sensitivity for SLEV detection. However, the assay needs to be evaluated in a clinical context and then integrated in routine laboratory. The SLEV diagnostic is required to ensure sensitive, specific and accurate identification of the viral agent and allow prompt surveillance action by health such as epidemiological monitoring and control measures these infections.

A-422

ZDC (Zika, Dengue and Chikungunya): The Performance of Kit for Multiplex Detection in Brazilian Laboratory

J. P. Silva, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Infections caused by arboviruses are considered as emerging infections of past decades and represent a worldwide public health problem. In Brazil, epidemics of arboviruses have already been described. Attempts have been made to develop less laborious, faster, and more sensitive diagnostic methods such as molecular multiplex methods. **Objective:** To evaluate the performance of commercial BioRad ZDC (Multiplex Assay for Molecular Detection of ZIKV, DENV and CHIKV) in Brazilian laboratory (Institute Hermes Pardini). **Methodology:** The real time RT-PCR protocol was performed according to the manufacturer´s instructions. Performance of kit was evaluated following: i) Sensibility: DENV, CHIKV and ZIKV positive samples; ii) Specificity: DENV, CHIKV and ZIKV negative samples; iii) Cross reaction: test with Yellow fever virus (YFV) commercial control; iv) Determination of detection limit (the range of 1.000 to 10 copies/μL); v) Intrassay and interassay precision; vi) Co-infection: panel-tests with concentrations of controls: CO1 (high concentration of DENV/ZIKV/CHIKV), CO2 (low concentration of DENV/ZIKV/CHIKV), CO3 (low and high concentration of DENV and ZIKV/CHIKV, respectively), CO4 (low and high concentration of DENV/ZIKV and CHIKV, respectively), CO5 (high and low concentration of DENV and ZIKV/CHIKV, respectively). **Results:** The clinical sensibility data showed 100% of concordance among the tests (n=8 DENV; n=21 ZIKV, n=15 CHIKV). In addition, the results suggested that ZDC kit presents high specificity and no cross reaction. The ZDC test had a limit of detection (LOD) of 10 copies/μL for CHIKV and ZIKV, 10 to 100 copies/mL for all DENV serotypes. The precision demonstrated optimal repeatability and reproducibility. The co-infection study is presented in Table 1. **Conclusions:** These results demonstrated that the ZDC kit has high sensibility and specificity and it could be used for molecular diagnostic of three arboviruses. Besides that, the multiplex technology has the potential to become a pratical, rapid and precise diagnostic test in clinical laboratories.

The co-infection study.

Coinfection	DENV	ZIKV	CHIKV
CO1	+ (Cq=29)	+ (Cq=29)	+ (Cq=26)
CO2	+ (Cq=35)	+ (Cq=37)	+ (Cq=33)
CO3	+ (Cq=33)	+ (Cq=29)	+ (Cq=33)
CO4	+ (Cq=33)	+ (Cq=37)	+ (Cq=26)
CO5	+ (Cq=30)	+ (Cq=36)	+ (Cq=33)

A-423

Characterization of Viral Genomic Mutation in Novel Influenza A (H7N9) Infected Patients: The Association between Oseltamivir-Resistant Variant and Prolonged Viral Shedding Duration

F. Yu[1], Q. Zhou[1], Z. Huo[2], S. Zheng[1]. [1]*Center of Clinical Laboratory, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China,* [2]*School of Medicine, Zhejiang University, Hangzhou, China*

Background: Since February, 2013, the human infection with a novel influenza A H7N9 virus has occurred in eastern China. It is important to detect the mutation of viral genes and analyzed the clinical features of the patients as well as the duration of viral shedding in relation to neuraminidase (NA) inhibitor resistance.

Methods: We collected clinical specimens from 31 hospitalized patients with laboratory-confirmed H7N9 infection between March 19 and May 31,2013 every day whenever available, and obtained NA, PB2, HA and M gene fragments by reverse-transcription PCR and sequenced them by Sanger method. We also analyzed the association of NA-R292K mutation confering NA inhibitors resistance and viral shedding duration.

Results: Of 31 patients identified, 7(22.6%) carried the substitution of R292K in NA, 30 (96.8%), 3 (9.7%) and 5 (16.1%) carried E627K, Q591K and D701N in PB2 respectively, 2(6.5%) carried both E627K and D701N in PB2. Of 26 patients identified, all harbored Q226L mutation and possessed only a single arginine (R) at cleavage sites in HA and carried S31N in M2. Among 7 NA-R292K mutated patients, 3 died, and 4 discharged. The days for patients starting oseltamivir treatment after symptoms onset between NA-R292K mutant and NA-R292 wild type had no significant difference (median days, 7 vs 6, P =0.374), but NA-R292K mutant patients had significantly longer duration of viral shedding than NA-R292 wild type after oseltamivir treatment (median days, 10 vs 5, P=0.022).

Conclusion: The mutations related with increasing the abilities of viral binding, adaptation and replication in human as well as anti-viral drug resistances have emerged in H7N9 gene fragments. The mutation of R292K of NA gene conferring to potential ability of oseltamivir resistance resulted in prolonged viral duration and poor outcome, and should be taken into consideration in the clinical management of infected patients.

A-424

Performance Evaluation of the Atellica IM HIV Ag/Ab Combo (CHIV) Assay

N. K. Padda[1], M. Anostario[1], C. Conarpe[1], B. Plouffe[1], C. Brown[1], G. A. Carter[1], E. Saharig-Romero[1], P. Guidon[1], Z. Bostanian[2], A. A. Mohammad[3]. [1]*Siemens Healthineers, Tarrytown, NY,* [2]*Research and Development Institute, Calabasas, CA,* [3]*Scott and White Memorial Hospital, Temple, TX*

Background: Transmission rates for HIV infection are driven disproportionately by individuals who are unaware of their HIV status. Early detection and knowledge of HIV status are important in reducing transmission rates, and appropriate care reduces morbidity and mortality. As a result, recent guidelines recommend that HIV screening be routinely included in patient care. The Atellica® IM HIV Ag/Ab Combo Assay detects the presence of HIV-1 p24 antigen and antibodies to HIV-1 (including group O) and HIV-2 in serum and plasma. This study evaluated the clinical performance of the Atellica IM HIV Ag/Ab Combo Assay, used to aid in the diagnosis of HIV infection and AIDS.

Methods: Precision was determined in accordance with CLSI document EP05-A3. Precision pools that included three HIV-1, two HIV-2, one HIV-1 group O, two HIV-1 p24, and five control levels were assayed on an Atellica® IM Analyzer in duplicate twice a day for 20 days at a single site. Human serum pools were aliquoted and frozen prior to the start of the study. Each testing day, new aliquots were thawed and assayed. The method comparison study included a total of 8811 samples from high-risk, low-risk, apparently healthy, and HIV-infected (HIV-1, HIV-2, group O, and p24 Ag+/Ab-) individuals and hospitalized patients at three sites. Samples were assayed on both the Atellica IM and ADVIA Centaur® XP systems using three different reagent lots, with approximately one-third of the specimens tested on each lot to determine the comparison of clinical specificity and sensitivity. The diagnostic sensitivity of the CHIV Assay was evaluated with 1366 HIV-positive samples, and specificity was determined by using 5962 low-risk or apparently healthy, 1008 high-risk, and 475 HIV-2–endemic population samples. The 95% confidence intervals were calculated using the Clopper-Pearson method.

Results: Precision results show the assay has repeatability %CV ranging from 1.3 to 2.2% and within-laboratory %CV ranging from 2.0 to 3.0%. On the Atellica IM Analyzer, all positive samples were reactive, resulting in 100% sensitivity (95% CI: 99.73–100.00%). The specificity in the low-risk population was 99.87% (95% CI: 99.74–99.94%), in the high-risk HIV-1 population 99.20 % (95% CI: 98.43–99.65%), and in the high-risk HIV-2–endemic population 99.79% (95% CI: 98.82–99.99%). On the ADVIA Centaur XP system, all HIV-positive samples were reactive, resulting in 100% sensitivity (95% CI: 99.73–100.00%). The specificity in the low-risk population was 99.87% (95% CI: 99.74–99.94%), in the high-risk HIV-1 population 99.09 % (95% CI: 98.29–99.58%), and in the high-risk HIV-2–endemic population 99.79% (95% CI: 98.82–99.99%).

Conclusions: The results of this study show that the Atellica IM HIV Ag/Ab Combo Assay is a precise and accurate fully automated qualitative method to simultaneously detect the presence of both HIV p24 antigen and HIV antibodies in human serum or plasma. The clinical performance of the Atellica IM CHIV Assay is comparable to that of the ADVIA Centaur CHIV assay.

A-425

Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry as a Rapid Method for Distinguish MRSA ST5-II-t311 and ST59-IV/V-t437

H. Kong. *First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: Methicillin-resistant *S. aureus* (MRSA) has emerged as a global pathogen in both hospital and community settings with multiple healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) clones disseminated internationally. Our goal is to evaluate the effectiveness of matrix assisted laser

desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for rapid identification of HA-MRSA and CA-MRSA clones. **Methods:** Ninety-six MRSA clinical isolates from Zhejiang region of China, were subjected to *spa* typing, SCC*mec* typing and multiple locus sequence typing (MLST) and all the strains were identified simultaneous by MALDI-TOF MS. Principal component analysis of mass spectrum and genetic algorithm statistics were performed on the main clones of MRSA by ClinProTools software. **Results:** The results of molecular typing showed that the main ST types were ST5 (34.38%), ST59 (25%), and ST239 (10.42%), and spa type was t311 (47.92%), t437 (31.25%), and t002 (6.25%). The most common popular clone types were ST5-II-t311 (33.33%) and ST59-IV/V-t437 (19.78%). The cross-validation of model t311 and t437 is 84.85% and the recognition capability is 96.31% by the genetic algorithm in ClinProTools software. As well,the cross-validation and recognition capability of model ST5 and ST59 is 98.51% , 100.0% respectively. Interestingly, the cross-validation and recognition capability of model ST5-II-t311 and ST59-IV/V-t437 both are 100.0%. Mass spectral peaks with mass-to-charge ratios of 6482, 6553, 5698, and 4498 m/z are the most prominent characteristic peaks for distinguishing ST5-II-t311 and ST59-IV/V-t437. **Conclusion:** MALDI-TOF MS can be used to quickly and accurately distinguish the two genotypes ST5-II-t311 and ST59-IV/V-t437 which representing HA-MRSA and CA-MRSA clone, respectively, providing help for clinical rapid diagnosis and treatment of community-related MRSA infections.

A-426

Stability of Hepatitis C Virus Serological and Molecular Markers Using ViveST Transportation System

A. Poe, M. Kodani, J. Drobeniuc, T. Mixson-Hayden. *CDC, Atlanta, GA*

Background: Laboratory diagnosis of hepatitis C virus (HCV) infection is based on the detection of antibodies to HCV (anti-HCV) followed by HCV RNA or HCV RNA testing alone. In order to assure quality testing, proficiency panels are provided to domestic and international partners, requiring costly shipments on dry ice in order to maintain the quality and integrity of samples. We evaluated dried tube specimen (DTS) and lyophilization as alternative methods to shipping proficiency testing panels at ambient temperature; while the methods enabled detection of anti-HCV, the small volume used in DTS volume was insufficient for the detection of HCV RNA, and lyophilization required costly equipment and was low throughput. As an alternative, ViveST™ cartridges can accommodate up to 1 ml of biological matrix and use air-drying for preservation of specimens.

Methods: We conducted a two-step assessment of ViveST™ cartridges' ability to stabilize markers of HCV infection in human plasma. In the first step, to maximize HCV RNA recovery yield and antibody detection, we optimized the elution protocol by assessing elution times and temperatures. Overnight elution of specimen increased the recovery yield of both markers when compared to the 10-minute elution protocol suggested by the manufacturer. As a second step, we assessed the stability of anti-HCV and HCV RNA for 2 weeks at multiple temperatures, including 25 and 45°C.

Results: ViveST™ cartridges preserved both anti-HCV and HCV RNA positivity for at least two weeks at 25 and 45°C. Additionally, we investigated whether cross-contamination occurs in ViveST during the serum drying process when the cartridges remain open overnight.

Conclusion: In summary, complex specialized equipment required for lyophilization precludes this method as an alternative for shipping of proficiency samples. Similarly, the DTS elution volume prohibited the recovery of HCV RNA; therefore it was deemed a poor alternative for shipping proficiency panels. ViveST™ cartridges offer a simple method of achieving the same goal of preserving the integrity of hepatitis C markers during shipping at ambient temperature.

A-427

Evaluation of the Nucleic Acid Detection Methods of CT/NG & Survey of the Infection in Population

L. Kan, X. Zhang, D. Xiong, H. Tang. *The Third affiliated hospital of ShenZhen university, shenzhen, China*

Background: To evaluate the method of real-time fluorescent quantitative PCR for detection of Nucleic Acid of chlamydia trachomatis (CT) and gonococcal gonococcus (NG). To investigate the infection of CT and NG in a massive population. **Methods:** Sequencing was performed to confirm the accuracy of RT-PCR method.The intra and inter-batch precision was checked by detecting one strong positive sample and one weak positive sample each batch of 5 times in a period of 4 days and the Ct value was used to describe the variation. The sample with the lower limit declared by the manufacturer were tested 20 times and the coincidence rate was judged. **Results:** The results of RT-PCR were in full concordance with that of sequencing. The inter-group and intra-group CVs of Ct value were 0.63% and 0.11% in strong positive chlamydia trachomatis sample, while the data was 0.54% and 0.27% in weakly positive chlamydia trachomatis sample respectively. The inter-group and intra-group CVs of Ct value were 0.79% and 0.32% in strong positive Neisseria gonorrhoeae sample, while the data was 1.12% and 0.63% in weakly positive Neisseria gonorrhoeae sample respectively. The coincidence rate of the sample with the lower limit declared by the manufacturer for 20 times was 100%.The positive rates of CT and NG were 9.53% and 2.08% respectively. There was no significant difference between male and female in CT positive rate (P>0.05). The positive rate of NG in males was significantly higher than that in females (P<0.05). The positive rate of two pathogenic microorganisms was higher in patients with ≤ 30 years of age. **Conclusion:** RT- PCR is a rapid, convenient, accurate and stable method for the detection of CT and NG. This study provide information for clinical diagnosis, prevention and treatment of CT and NG infection.

A-428

Prevalence & Genotyping of Human Papillomavirus Infection in Female Patients in Shenzhen Luohu District

L. Luo, X. Xu, D. Xiong, H. Mo, X. Zhang. *The Third affiliated hospital of ShenZhen university, shenzhen, China*

Background: To investigate the genotypes distribution of 26 common human papillomavirus(HPV) and its prevalence among different age groups in Shenzhen Luohu People's Hospital.In order to provide the reference basis for the prevention and treatment of HPV infection and cervical cancer. **Methods:** Retrospctively analyzed the HPV genotyping results of 8071 females who visited in Shenzhen Luohu People's Hospital from January 2016 to January2017. According to different age, these cases were divided into four groups: <20,20~34,35~49,≥50. Prevalence and genotyping of human papillomavirus infection was analyzed by using SPSS17.0. And Compared distribution characteristics of different genotypes. **Results:** 1467 cases of HPV positive samples were detected in 8071 cases, and the prevalence was 18.18%. 1467 cases of positive samples included 1086 cases of single HPV infection and 381 cases of multiple HPVs infection, the prevalence of which were 13.46% and 4.72%. The highest infection rate was the first group(< 20 years old, 45.83%), followed by the second group(20~34 years old, 20.46%), and the lowest infection rate was 14.98%(35~49 years old). High-risk HPV-16, 52, 58 and low-risk HPV-6 were the most common genotype infection all ages, especially high-risk HPV-16. **Conclusions:** The HPV infection tends to younger age in Shenzhen Luohu People's Hospital. The study showed a "U" shaped distribution of age-specific prevalence of HPV. The top four genotypes were HPV-16, 52, 58 among high-risk HPV infection. The age and subtypes distribution of HPV infection were unique in this region.And this can provide important clinical basis for the early diagnosis,the prevention and the use of HPV vaccine of the early cervical cancer .

A-429

Utility of C-Reactive Protein and Leukocyte Esterase in Synovial Fluid in the Diagnosis of Septic Arthritis

N. Stanzione, P. Ravelo, L. Song. *UCLA, Los Angeles, CA*

Background: Septic arthritis (SA) is commonly caused by bacteria infection of a joint. Rapid diagnosis and treatment is paramount to prevent joint damage. While analysis of joint fluid for bacteria and white blood cell (WBCC) is useful in the diagnosis of SA, the sensitivity is relatively low. Recent studies have examined additional markers including C-reactive protein (CRP) and leukocyte esterase (LE) within synovial fluid to increase the diagnostic accuracy. Often CRP can't be measured from synovial fluid on an automated platform due to its high viscosity. We developed a method to reduce the viscosity and assess the utility of CRP and LE in synovial fluid in the diagnosis of SA.**Methods:** Eighteen serum and 15 synovial fluid samples were treated with hyaluronidase, and CRP results were obtained on the Dimension Vista 500 (Siemens Healthcare Diagnostics, Tarrytown, NY). The CRP results in the hyaluronidase treated versus untreated serum samples yielded a slope of 0.98 with no intercept. The CRP results from the 15 hyaluronidase treated versus untreated synovial fluid samples yielded a slope of 1.04 with no intercept. These studies proved the validity of CRP results in hyaluronidase treated samples.Thirty-one synovial fluid samples were tested for LE, CRP, WBCC with neutrophil percentage. Viscous synovial fluid samples were treated with hyaluronidase (Sigma-Aldrich Catalog# H3506-500MG) to degrade hyaluronic acid. CRP was measured with the Vista 500. WBCC and neutrophil percentage were measured on the Sysmex XN 9000 (Sysmex America, Irvine, CA). LE was measured with IChem VELOCITY Urinalysis Instrument (Beckman

Coulter Diagnostics, Brea, California). Using a scoring system[1], results of WBCC >3000/cm^3, percent neutrophils >80%, CRP >0.69 mg/dL, and LE ≥2+ in synovial fluid were given scores of 3, 2, 1, and 3 respectively. The validity was determined in reference to clinically determined SA.**Results:** In the 11 cases of clinically positive for SA, the average CRP was 5.66 mg/dL, and the average WBCC was 35,691/cm^3 with 90% neutrophils. LE results were obtained in 7 of the positive cases, all showing at least "trace" positivity. In the 20 negative cases, the average CRP was 1.22 mg/dL, and the average WBCC was 9,823/cm^3 with 41% neutrophils. LE results were obtained in 19 of the negative cases, 14 of which had negative results (73.6%). The SA cases had significantly higher LE (p=0.004), CRP (p=0.004) and WBCCs (p=0.003). Synovial fluid with a WBCC >10,000 per cm^3, CRP ≥2 mg/dL, and positive LE yielded a sensitivity of 71.4%, specificity of 89.5%, and diagnostic accuracy of 84.6%.Of the 7 positive SA cases evaluated by the scoring system, the average was 6.4. Of the 19 negative cases, the average score was 1.5. With a cutoff of 4 points, the sensitivity and specificity of SA is 100% and 78.9%.**Conclusion:** Synovial fluid viscosity can be reduced with hyaluronidase without affecting CRP testing accuracy. Using a scoring system, synovial fluid LE and CRP can add value to the workup of SA.**Reference:**

1. Parvizi J, et al. The 2018 Definition of Periprosthetic Hip and Knee Infection: An Evidence-Based and Validated Criteria. J Arthroplasty. 2018;33(5):1309-1314.

A-430

Performance Evaluation of the Atellica IM HIV 1/O/2 Enhanced (EHIV) Assay

S. Seck[1], M. Anostario[1], C. Conarpe[1], B. Plouffe[1], C. Brown[1], R. Fonstad[1], E. Saharig-Romero[1], P. Guidon[1], A. A. Mohammad[2], Z. Bostanian[3]. *[1]Siemens Healthineers, Tarrytown, NY, [2]Scott & White Memorial Hospital, Temple, TX, [3]Research and Development Institute, Calabasas, CA*

Background: HIV remains a serious public-health issue in the world. There are nearly 37 million people globally living with HIV/AIDS virus, and 25% are unaware of their virus status. HIV testing is crucial for improving the health of those living with the disease by allowing them to initiate treatment as early as possible. Testing also helps reduce transmission of HIV and prevent new infections. The results of precision and clinical performance evaluations of the Atellica® IM HIV 1/O/2 Enhanced (EHIV) Assay are presented for this study.

Materials and Methods: Precision was determined per CLSI EP05-A3, using 10 HIV-positive serum samples tested on an Atellica® IM Analyzer with the EHIV Assay. Each sample was tested in duplicate twice a day for 20 days at one site. These samples included four HIV-1, four HIV-2, two contrived samples with HIV type O, as well as four controls. Serum pools were aliquoted and frozen prior to the start of the study. New aliquots were thawed and used for each testing day. Within-lab imprecision and repeatability estimates were calculated using a nested ANOVA model. The mean, SD, and %CV for each precision component were computed.

Clinical performance was estimated for both the Atellica IM EHIV Assay and the ADVIA Centaur® EHIV assay. The clinical sensitivity was evaluated using a total of 1295 individuals positive for HIV, including HIV-1, HIV-1 group O, HIV-2, pregnant women, and pediatric populations. The clinical specificity study tested 7442 specimens, including low-risk specimens (apparently healthy pregnant women, pediatrics, and hospital patients); high-risk specimens (injection drug user, multiple transfusions, sexually transmitted disease patients); and individuals from HIV-2–endemic areas. These samples were tested at three sites using two reagent lots, where one-half of the population was tested with each lot. Clinical sensitivity and specificity were calculated and presented alongside the respective 95% confidence intervals (Clopper-Pearson).

Results: Precision results for the EHIV panel show that the assay has a %CV ranging from 2.5 to 4.3% for repeatability and 2.8 to 5.5% for within-laboratory imprecision. On the Atellica IM Analyzer, the clinical sensitivity in the HIV-positive population was 100% (95% CI: 99.72%–100.00%). The clinical specificity in the low-risk population was 99.90% (95% CI: 99.78–99.96%), in the high-risk population (HIV-1) 99.60% (95% CI: 98.97–99.89%), and in the HIV-2–endemic area population 100% (95% CI: 99.22–100.00%). On the ADVIA Centaur XP system, the clinical sensitivity in the HIV-positive population was 100% (95% CI: 99.71–100.00%). The clinical specificity in the low-risk population was 99.90% (95% CI: 99.78–99.96%), in the high-risk population (HIV-1) 99.59% (95% CI: 98.96–99.89%), and in the HIV-2–endemic area population 100% (95% CI: 99.22–100.00%).

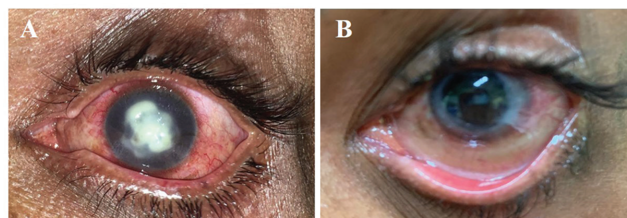
Conclusions: The results show that the fully-automated Atellica IM EHIV Assay has good clinical performance with high precision. The clinical performance of the Atellica IM EHIV Assay is comparable to the clinical performance of the ADVIA Centaur EHIV assay.

A-431

Severe Fungal Keratitis in Immunocompetent Patient: Case from Banal Attitude

S. P. Bandeira[1], F. Nepomuceno Neto[2], M. C. G. CASTELO[1], T. A. C. Palhano[1], F. M. P. Ventura[1], R. R. Araujo[1], L. L. A. Cavalcante[1], G. A. Campana[3]. *[1]DASA, FORTALEZA, Brazil, [2]Brazilian Society Of Ophthalmology, FORTALEZA, Brazil, [3]DASA, São Paulo, Brazil*

Background: Infectious keratitis caused by fungus is a serious eye disease that is difficult to manage due to limited available therapeutic arsenal and frequent late diagnosis. Objective: Describe a case of severe unilateral keratitis caused by *Fusarium solani* complex in an immunocompetent patient. Case report: A 45-year-old male patient, with no comorbidities, had intense eye irritation in left eye. Patient reported he was riding a motorcycle without eye protection when he came in contact with grains of sand from a heavy vehicle. After two days, he presented photophobia with conjunctival hyperemia and corneal ulcer was diagnosed. Topical treatment was given with moxifloxacin and prednisone drops in high doses, without clinical improvement. After 33 days, clinical status evolved with ocular perforation (Figure 01A). A penetrating corneal transplant was performed associated with anterior chamber lavage with vancomycin. After about 14 days, a recurrence of infection was observed in the corneal receptor bed. Microbiological exams were performed on silk thread passed by the whitish corneal stroma. After three days of incubation, it was observed growth of colony with cottony texture in white tone that acquired pink gray hue. Micromorphological analysis demonstrated perpendicular conidiophore with numerous fusiform, slightly curved, bi and multissepted macrofialoconids which are characteristic of genus *Fusarium*. Isolate was submitted to MALDI-TOF technique, identifying *Fusarium solani* complex with 99.9% accuracy. Topical corticosteroids were discontinued and treatment with amphotericin B eye drops was instituted for 5 days. Patient presented toxicity to eye drops, leading to suspension of drug. It was initiated 5% pimaricin eye drops. Slow improvement associated with risk of scleral infection was decisive to indicate hospitalization for intravenous medication. Patient was hospitalized for 14 days in use of voriconazole, presenting clinical improvement (Figure 01B). At the moment, he is under ambulatory follow-up, complementing antibiotic treatment in a great improvement curve.



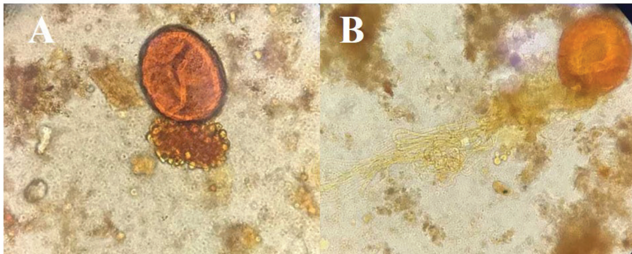
A-432

Urbanorum spp.: A New Public Health Problem in Brazilian Northeast?

S. M. A. R. ARAUJO[1], A. J. DOMINICI[1], M. S. SILVA[1], V. C. F. CASTRO[1], R. M. MAIA[1], M. F. SANTOS[1], J. A. F. BRAGA[1], L. L. A. Cavalcante[2], M. C. G. CASTELO[2], S. P. Bandeira[2], G. A. Campana[3]. *[1]DASA, SÃO LUÍS, Brazil, [2]DASA, FORTALEZA, Brazil, [3]DASA, SÃO PAULO, Brazil*

Parasitic enteric diseases are among major public health problems in the world population and contribute to high morbidity and mortality rates, affecting 3.5 billion people worldwide, especially in developing countries. The parasite Urbanorum spp was described for first time by Francisco Santamaría (1991) and represents another etiological agent of these intestinal pictures. This microorganism affects individuals of all ages, sex and social classes, causing more severe conditions in populations younger than 5 years. Objectives: The aim of this study was to evaluate frequency of Urbanorum spp. in clinical samples received in a private laboratory of clinical analyzes from the city of São Luís and São José de Ribamar (MA) in Brazilian Northeast. Another objective was to draw an epidemiological profile of patients. Metodology: Review of laboratory data from January to December, 2018. Results: During this study period, 28,330 analyzes were performed on faecal samples. The clinical specimens were analyzed by light microscopy (40x), stained by Lugol tecnique. Of total samples, 149 (0.52%) demonstrated presence of pathogen characterized by yellowish, rounded structures (80 to 100 μm), double outer membrane with pores and emission of filaments similar to pseudopodia (Figure 01 A/B). There was no trend of occurrence by age group, however, 64% of the cases occurred in the female sex. Discusssion: No

records of this parasite were observed in years prior to the study in the laboratorial service in question. As analysis platform and laboratory staff were not modified, we conclude that there was a significant increase in detection of cases in the population, which may reflect an increase in contamination index. New studies will be needed to evaluate behavioral issues and clinical attributes of these cases, however we can certainly be facing a new public health problem in this region.



A-433

Comparison of the Aptima HBV Quant Assay in the Automated Panther® System with the Abbott Realtime HBV Assay in the Quantification of Hepatitis B Virus

D. R. Orta Jacobo, N. d'Empaire, E. Carmenatis, R. Guevara. *BioCollections Worldwide Inc, Miami, FL*

Background: Hepatitis B virus (HBV) is one of the most common infectious diseases affecting more than 350 million people worldwide. HBV is a major cause of chronic liver disease with an increased risk of cirrhosis and hepatocellular carcinoma. Monitoring HBV DNA levels, prior to and during treatment, aids in assessing response to treatment and the emergence of resistance. Nowadays there are multiple assays and platforms available to detect and quantify HBV. The objective of this study was to compare the Aptima HBV Quant Assay, released by Hologic, Inc®, with the Abbott Molecular Realtime HBV assay. The Aptima HBV Quant assay is an in vitro dual-target nucleic acid amplification test for the quantitation of HBV DNA in human plasma and serum on the Panther™ system. The Abbott Realtime HBV assay is an RT-PCR test run on the m2000 system. **Methods:** One hundred twenty nine plasma specimens, seventeen negative and one hundred and twelve positives for HBV, previously tested on the Abbott m2000 platform, were included in this study to evaluate sensitivity and specificity performance. In addition, to corroborate specificity, some of the negative specimens were positive for Human Immunodeficiency Virus, Hepatitis C Virus, Hepatitis A Virus and *Toxoplasma gondii*. One hundred and ten specimens, with known viral loads, were used to test the quantitative performance. Precision was tested using an HBV positive specimen repeated fifteen times in different runs. The specimens were assayed using the Aptima HBV Quant Assay on the Panther System following the manufacturer's instructions. Results obtained were compared using the EP Evaluator program. **Results:** HBV Viral Loads were analyzed through both methods to determine whether the methods were equivalent within the allowable total error of 1 $\log_{10}$ cop/mL. One hundred and ten positive specimens were compared over a range of 1.00 to 9.00 $\log_{10}$ IU/ml, the test passed with a correlation of 0.98. The difference between the methods was within allowable error for 105 of the 110 specimens (95.5%) tested. For the qualitative performance, results showed a positive agreement of 98.2% and a negative agreement of 100%. Two out of 112 specimens showed not detected results on the Panther system. Both samples had detectable, but not quantifiable viral loads (<10 IU/mL) on the Abbott m2000 system. This can be explained because 10 IU/mL is within the allowable error at low viral load ranges for the Panther system. Specificity of the Aptima HBV Quant assay showed a not detected result for all specimens, which represents a 100% agreement. For the precision study the EP Evaluator results showed a mean of 6.126 log (10) IU/mL with a standard deviation of 0.051. These results were within 2 SD ranges. **Conclusion:** The Aptima HBV Quant assay demonstrated high efficiency, accuracy and reproducibility for the detection and quantification of HBV, with a performance equal to that of the Abbott Realtime HBV assay. The random access to the Panther system along with the full automation, the ease of use, and the reduction in hands-on time, makes the Aptima HBV Quant assay a good option to detect and quantify HBV.

A-434

Phenotypic Susceptibility Profiles and Antimicrobial Resistance at a Tertiary Teaching Hospital in the Dominican Republic

J. J. Rodriguez Abreu[1], A. J. Mena Lora[2], J. de Lara[1], C. Blanco[1], G. Esparza[3], S. C. Bleasdale[2]. [1]*CEDIMAT, Santo Domingo, Dominican Republic,* [2]*University of Illinois at Chicago, Chicago, IL,* [3]*Universidad Javeriana, Bogota, Colombia*

Background:

Antimicrobial resistance (AMR) is a rising global challenge. The burden of AMR can vary by geography and healthcare settings. Antimicrobial use and contact with healthcare facilities are risk factors for AMR and contribute to higher rates of resistance in hospitalized patients. There is a paucity of data on AMR susceptibility patterns in developing nations. We seek to define AMR in at a tertiary hospital in the Dominican Republic (DR).

Methods:

This is a retrospective review of susceptibility patterns in clinical isolates at a tertiary hospital in the DR. Susceptibility data from all inpatient clinical cultures were collected from January to December 2017. Automated susceptibility testing (Vitek®2, bioMérieux) was performed for routine susceptibility testing.

Results:

A total of 1802 positive cultures reviewed. The most common organisms were *Escherichia coli* (30%), *Klebsiella pneumoniae* (9%) and *Staphylococcus aureus* (7%). The most common sources were urine (44%) and blood (21%). Phenotypic patterns consistent with extended spectrum beta lactamase (ESBL) were present in 42% of *E coli* and 50% of *K pneumoniae*. Vancomycin resistance (VRE) was present in 6% of 50 *Enterococcus* isolates. Of 120 *S aureus* isolates, methicillin resistance (MRSA) was found in 50%. Susceptibility patterns for gram negative bacilli are in Table 1.

Table 1. Susceptibility patterns for Gram-negative bacilli (%)

Organ-isms (n =1802)	Ampi-cillin-Sul-bac-tam	Cefa-zo-lin	Oxacillin	Cefta-zidime	Ceftri-axone	Cefe-pime	Cipro-floxacin (FQ)	Genta-micin	Imi-penem	Piper-acillin-tazo-bactam (PTZ)	Vanco-mycin
E.coli (n =541)	55	66		58	58	58	40	68	100	97	
K.pneu-moniae (n =173)	46	47		50	48	48	53	66	100	85	
P.aeru-ginosa (n =109)				95		95	86	92	87	90	
A.bau-mannii (n =30)	93			74		88	63	70	93	88	

Conclusion:

The prevalence of AMR in hospitalized patients in the DR is high. *Enterobacteriaceae* had high levels of cephalosporin resistance suggesting ESBL-producing organisms, with rates above 42% in *E coli* and above 50% in *Klebsiella.* Quinolone resistance is above 50% for *Enterobacteriaceae*. MRSA rates are comparable to developed nations. *A. baumannii* and *P aeruginosa* remain susceptible to most agents. Local resistance patterns for *Enterobacteriaceae* limit the use of common beta-lactams and oral agents. Further genotypic studies may be needed to understand the epidemiology of local resistance genes.

A-435

Frequency of Contamination with Clinical Specimens during Laboratory Testing: Evaluation of Laboratory Workers, Surfaces, and Automated Instrumentation

C. W. Farnsworth, M. A. Wallace, A. M. Gronowski, C. D. Burnham, M. L. Yarbrough. *Washington University, St. Louis, Saint Louis, MO*

BACKGROUND: Every specimen processed by the clinical laboratory is potentially infectious. However, data regarding the risk points for contamination of the clinical laboratory environment or staff during routine testing are scarce. Our objective was to assess laboratory contamination with clinical specimens during routine sample analysis in automated clinical chemistry and microbiology laboratories.

METHODS: Non-pathogenic MS2 virus was added to urine and plasma specimens to a final concentration of $1.0x10^7$ PFU/mL. To assess gross contamination, a fluorescent powder was applied to the exterior of the sample tubes. Samples were transported by pneumatic tube to the laboratory and handled by experienced personnel according to standard operating procedures. Urine samples were processed and analyzed on the BD Kiestra InoqulA and TLA (n=3). Plasma samples were processed on a Cobas 8100 (Roche) and analyzed on Cobas ISE, c502, e602, and c702 modules (n=3). Fluorescence contamination on surfaces, instrumentation, and personnel (n=9) was visualized

using UV light. High-risk areas for MS2 contamination were swabbed and assessed using RT-PCR for MS2.

RESULTS: Fluorescence was observed on 51/173 (29%) of surfaces and personnel sampled (Table). Fluorescence was routinely noted on gloves of personnel (23/26), mouse/keyboard (6/12), and loading/centrifuge racks (12/12). In contrast, no fluorescence was observed on the hands/wrists/face of personnel (0/27) or on mechanical portions of the automated instrumentation such as probes, and decappers (0/33). Of 28 areas sampled on the Cobas 8100 and chemistry analyzers, none were positive for MS2 contamination.

CONCLUSIONS: Our findings support that handling contaminated specimen containers can result in contamination of high-touch laboratory surfaces and represents a potential point of risk; specific decontamination protocols should be established to mitigate this risk. Analysis of infectious specimens on automated Chemistry and Microbiology analyzers elicited a low likelihood of instrument contamination.

Table Frequency of fluorescent contamination

	Surface Sampled	Cobas 8100	Cobas Chemistry Analyzers	BD Kiestra	Combined (%)
Lab worker / receiver	Gloves	8/8	N/A	15/18	23/26 (88%)
	Lab Coat Cuff	0/8	N/A	2/18	2/26 (8%)
	Hands/Wrists/Face	0/10	N/A	0/27	0/37 (0%)
Receiving Area	Mouse / Keyboard	3/6	N/A	3/6	6/12 (50%)
	Specimen Holding Rack	1/2	N/A	2/3	3/5 (60%)
	Phone	N/A	N/A	1/1	1/1 (100%)
Instrumentation	Loading/ Centrifuge Racks	3/3	3/3	6/6	12/12 (100%)
	Mechanical Arms	0/6	N/A	0/3	0/9 (0%)
	Decapper	0/3	N/A	0/3	0/6 (0%)
	Waste	2/3	N/A	0/6	2/9 (22%)
	Probes / Pipettor	N/A	0/15	0/3	0/18 (0%)
	Drip Tray	0/3	N/A	0/3	0/6 (0%)
	Interactive Surfaces *	N/A	N/A	2/6	2/6 (33%)
Total Events					**51/173 (29%)**

*Includes a barcode scanner and a handle to open/close instruments for loading

A-436

A Possible Algorithm for Diagnosis and Screening of *Clostridium difficile*

D. Zauli[1], C. C. Silva[2], F. Brito[3]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Microbiology Division), Belo Horizonte, Brazil, [3]Hermes Pardini Institute, Belo Horizonte, Brazil*

Background: *Clostridium difficile*, an anaerobic toxigenic bacterium, causes a severe antibiotic-associated diarrhea infection (CDI) that leads to significant morbidity and mortality worldwide. The bacteriological diagnosis of this disease is based on the detection of toxins B and/or A of *C. difficile* in stool specimens. To improve the sensitivity and speed of diagnosis, a strategy based on a two-step algorithm of is recommended by guidelines from the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). The first step is the glutamate dehydrogenase (GDH) assay followed by toxin or PCR plus toxin for identifying toxin-producing *C. difficile* isolates. However, continuing controversy still exists regarding the best option for the diagnosis and screening this of pathogen. **Objective:** To design and propose an algorithm of *C. difficile* diagnosis using test for detection of GDH (Biomerieux VIDAS GDH) and molecular detection (Cepheid Xpert *C. difficile*) in stools in which the results of Toxin A/B are previously known. **Methodology:** A total of 168 fecal samples from patients with suspect of CDI were tested simultaneously by VIDAS GDH and GeneXpert. For VIDAS GDH assay the samples were grouped according to the validation protocol: Group 1: 40 toxin A/B positive samples; Group 2: 60 toxin A/B indeterminate samples; Group 3: 68 toxin A/B negative samples. Genexpert *C. difficile* assay was done on 38 samples as follow: Group 4: 12 toxin A/B and GDH positive samples; Group 5: 10 toxin A/B and GDH negative samples; Group 6: 6 toxin A/B negative and GDH positive samples; Group 7: 10 toxin A/B indeterminate and GDH positive samples. All assays were performed according to the instructions of the manufacturer. **Results:** Twenty six (65%) and fourteen (35%) of 40 toxin A/B positive samples were GDH positive and negative, respectively. Of toxin A/B negative samples 90% were GDH negative (61/68). *C. difficile* was detected by Genexpert in eight (67%) of 12 toxin A/B and GDH positive samples. There was a concordance of 100% with toxin A/B and GDH negative samples. In GDH positive and toxin A/B negative or indeterminate samples there were a positivity rate of 37.5% (6/16). **Conclusions:** The molecular finding was unexpected because positive samples for GDH and toxin A/B are considered positive for *C. difficile*, and the sensitivity of the molecular test is high. Perhaps, this is due to the conservation of the sample due to the selection and test design of samples used in the study The results obtained with VIDAS GDH assay suggest the use of GDH and toxin A/B detection simultaneously for all patients, and molecular detection (Genexpert) for the cases whose results are discordant as a step in algorithm of *C. difficile* detection. This study provides a three-step algorithm (Toxin → GDH → Xpert) as a better choice for CDI diagnostic.

A-437

Improvement of Non-Amplification Nucleic Acid Method to Detect Pathogenic Genes using dA Probe-polyT Probe System

S. Watanuki[1], M. Komatsu[1], K. Ito[1], R. Takeuchi[1], K. Nakaishi[1], T. Yoshimura[2], E. Ito[3], S. Watabe[1]. *[1]TAUNS LABORATORIES, INC., Izunokuni, Shizuoka, Japan, [2]Health Sciences University of Hokkaido, Ishikari, Hokkaido, Japan, [3]Waseda University, Shinjuku, Tokyo, Japan*

Background: Genetic testing methodologies such as PCR system have been widely adopted in the clinical field. However, these methods require expensive and specialized apparatus, reagents and advanced skills. Recently, we have developed a non-amplification nucleic acid detection method to overcome the drawbacks of PCR. This method is based on hybridization and an enzyme cycling system constructed by a dehydrogenase (3α-hydroxysteroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androsterone derivatives). In the present study, we aimed to further develop a higher sensitive method to detect target genes (any kind of pathogenic genes) without gene amplification.

Methods: An optimal repeated sequence in a target gene region was selected by sequence analysis. A polyT (100 nt)-linked probe (polyT probe) for the repeated sequences of the target gene and a FITC-labeled Oligo (6 nt) dA probe (dA probe) were prepared. A biotinylated probe for the target gene was also prepared and used to immobilize to an avidin-coated microplate. The target genes, polyT probes and dA probes were added into a test well. A large amount of dA probes hybridized with target genes via polyT probes. These FITC-labeled dA probes were measured by a thio-NAD cycling system with ALP-conjugated anti-FITC antibody. In the present study, only the dA probes hybridized with polyT probes was measured. In addition, when the Influenza A hemagglutinin (HA) region was used as a target model, the expected sensitivity using our method was calculated.

Results: To estimate the expected sensitivity of our new nucleic acid assay, we first evaluated the hybridization ratio of dA probes to polyT probes. 200 nM or 10 nM dA probes were hybridized with 10 nM polyT probes. The average absorbance using 200 nM dA probes and 10 nM dA probes was 0.331 Abs and 0.035 Abs, respectively, suggesting that the hybridization ratio of 200 nM dA probes was 9.5-fold higher than that of 10 nM dA probes. Then, we analyzed the nucleotide sequence of the influenza A HA region to determine an optimal repeated sequence. The optimal repeated sequence was found to be repeated 30 times. That is, when our new system is used, the hybridization ratio is expected to be 285-fold higher in the dA probe-polyT probe assay than the our previous method, suggesting that the genes can be detected at approximately 10 copies.

Conclusion: We demonstrated that our new nucleic acid assay can detect target genes with high sensitivity and user-friendly without gene amplification. The efforts of reducing nonspecific reaction and improving hybridization efficiency may further increase the sensitivity of the present assay.

A-438

Molecular Epidemiology of Hospital Acquired *Clostridium difficile* Infection in Two Regions of Zhejiang and Evaluation of Its Clinical Predicting Model

L. Wang, X. Wang. *Hangzhou First People,s Hospital, Hangzhou, China*

Background:

Clostridium difficile infection (CDI) as a major cause of antibiotic-associated diarrhea has been reported in China recently. Detailed CDI symptoms are, however, not disclosed in Chinese people, and no nomogram models are available to predict whether diarrhea samples need C. difficile test or not.

Methods:

We performed a one-time cross-sectional study in Hangzhou First People's Hospital Healthcare Facility Group (HFPH) and Lishui Second People's Hospital (LSPH) in Zhejiang from June 2015 to September 2017. Stool specimens collected from consecutive hospitalized patients with diarrhea were cultured for C. difficile and the isolates were analyzed for the presence of toxin genes, multilocus sequence analysis (ST) and antimicrobial susceptibility testing. CDI severities were defined by IDSA/SHEA guidelines according to blinded medical record review.

Results:

A total of 190 C. difficile isolates were recovered from 1,250 patients (15.2%). Of them, 110 (57.9%) had both tcdA and tcdB (A+B+), 71(37.4%) were negative for tcdA and positive for tcdB (A-B+), and 9 (4.7%) had no toxin genes (A-B-). ST37 (19.5%) and ST54 (16.8%) were the dominant genotypes. CDI severity is generally mild to moderate, and tcd A-B+ was the dominant genotype with severity CDI ($P < 0.001$). All the isolates were completely susceptible to vancomycin and metronidazole, and the antibiotic resistance of Clindamycin in LSPH (100.00%) was higher than HFPH (84.40%)($P<0.001$). Clinical data of 1250 patients with diarrhea were retrospectively analyzed. Multivariate Logistic regression analysis revealed that, Neutrophils with less than 70%, Lymphocyte with more than 40%, RBC with less than 5.5×1012/L, Hb with less than 160 g/L, and PLT more than 300×109/L in blood were the independent clinical indicators for CDI($P<0.05$). All the patients were randomly divided into the model establishment group (n=1000, 80%) and validation group (n=250, 20%). The nomogram mode was established with a C-index of 0.727, and the C-index of the validation group was 0.719, indicating that the nomogram model was qualified to predicting CDI in patients with diarrhea.

Conclusion:

CDI has been undoubtedly a problem in hospitals in China, and the tcd A-B+ genotype should be emphasized in CDI control and prevention. The monogram model could play a role on guiding clinician to predict CDI through common clinical indicators and driving clinical CDI diagnosis in China.

A-439

Performance of Identification of Organisms in Positive Blood Cultures by Mass Spectrometry with Short-Term Incubation

V. A. C. Castro-Lima, L. M. B. Correa, A. S. Brito, E. J. U. Kusano. *Diagnostico das Americas - DASA, São Paulo, Brazil*

Background: Blood-stream infection (BSI) is a severe disease with high mortality. It can occur in the context of community or nosocomial-acquired infections. Use of antibiotics is the cornerstone in the management of this infection. Identification of causative organism is essential in the management of antibiotics in BSI, it can guide the chosen of the ideal drug, avoiding excessive use of antibiotics and emergence of bacterial resistance. Standard microbiologic methods for identification of organisms are slow and depend of a period of 24 hours of incubation for colony growing, delaying adjustment of antibiotic therapy in clinical practice.

Methods: It was a prospective study that evaluated all blood culture samples forwarded to microbiology lab from patients with BSI suspected from 30 private hospitals in the state of São Paulo-Brazil, during two weeks of July 2018. The blood samples were collected in standardized BD BACTEC aerobic and anaerobic bottles and incubated in BACTEC FX (BD) until five days to detect bacterial growing. All positive samples were spread in plates with blood-agar, chocolate-agar and MacConkey and incubated at 35°C. The plates were observed at 3, 6 and 9 hour of incubation, if a minimal growth was observed a sample of colonies was obtained using a sterile loop to identification by Matrix Assisted Laser Desorption Ionizated-Time of Flight (MALDI-TOF). Colonies from all plates were repeatedly obtained after 24 hour of incubation using the same method, as the standard comparator.

Results: Among 260 positive blood cultures, 227 (85.8%) have identified organisms with a period of incubation less than 24 hours. There were identified 107 gram-negative organisms, 101 (94.4%) with less than 6 hours of incubation. One (0.9%) discordance was observed in gram-negative organisms: Escherichia coli was identified using short-term incubation whereas Providencia stuartii with 24 hours incubation. Among 111 gram-positive organisms, 99 (89.2%) were identified with a period of incubation less than 6 hours. There were 7 (6.3%) discordances in these organisms comparing with 24 hours incubation, four of them agreed with the genus, but not species, all of them coagulase-negative Staphylococcus species. Two isolates identified as Enterococcus faecalis and one as Streptococcus pneumoniae with short-term incubation were identified as S. pneumoniae, Stahyococcus aureus and Staphylococcus epidermidis using 24 hours incubation, respectively.

Conclusion: We have observed a high frequency of identification of organisms using MALDI-TOF after a short-term incubation of positive bood culture specimens. Majority of organisms were identified with less than 6 hours of incubation, with a higher frequency of gram-negative being identified in this period. There were more discordances among species, comparing with 24 hours incubation, in gram-positive organisms, however most of them agreed the genus and were coagulase-negative Staphylococcus species. Identification of organisms from positive blood cultures is faster and accurate using short-term incubation and can provide a relevant information for clinical decision in a severe infection such as BSI.

A-440

Evaluation of the Role of Long Non-coding RNAn343017 as a Promising Novel Biomarker in Patients with Tuberculosis

S. Guo, M. Wang, H. Tan, L. Xiao, W. Yao, B. Ying. *West China Hospital, Sichuan University, Chengdu, China*

Background: Tuberculosis (TB) remains a global public health problem, and improvements in timely and effective diagnosis are urgently needed. Long non-coding RNAs (lncRNAs) are novel transcripts that may play important roles in many diseases, including tuberculosis diseases. Our study aimed to investigate lncRNA n343017 for TB early differential diagnosis. **Methods:** LncRNA n343017 was selected from lncRNAs microarrays of 6 TB patients and 3 healthy controls. The gene expression of n343017 was analyzed by using qRT-PCR in 1739 peripheral blood mononuclear cell (PBMC) samples including 789 active tuberculosis patients (TB group), 638 healthy controls (HC group) and 312 latent tuberculosis infection individuals (LTBI group). Receiver operating characteristic (ROC) curves were constructed to explore diagnostic values. **Results:** n343017 expression was obviously downregulated in TB patients [TB vs HC vs LTBI: 0.49(0.14-1.12) vs 1.06(0.58-1.66) vs 0.99(0.47-1.96), $P = 4.932*10^{-4}$]. Compared with TB group and HC group, a cutoff level of 0.64 was set for n343017 expression to diagnose TB cases achieving a sensitivity of 96.45%, specificity of 67.87% with an area under the curve (AUC) of 90.22%. In TB group and LTBI group, the AUC was 69.45%, and cutoff value was 0.29 for TB disease differential diagnosis with a sensitivity and a specificity of 62.25% and 66.93% respectively. However, n343017 was not independently associated with disease-specific prognosis prediction. **Conclusion:** n343017 may serve as a potential diagnostic biomarker for tuberculosis timely diagnosis, and larger validation studies with different populations are warranted to confirm these findings.

A-441

Prevalence of Carbapenem Resistant Gram-Negative Agents in Blood Cultures in the State of São Paulo-Brazil

V. A. C. Castro-Lima, L. M. B. Correa, C. A. Santos, E. J. U. Kusano. *Diagnostico das Americas - DASA, São Paulo, Brazil*

Background: Gram-negative organisms are important causative agents of healthcare associated-infections infections (HAI), including bloodstream infections (BSI). Carbapenems agents are antibiotics with a broad spectrum of activity against bacteria and are widely used for BSI treatment in hospitals. In the last years it is being observed an emergence of multidrug resistant gram-negative organisms, including carbapenem-resistance, worldwide, with an impact in the antibiotic management and in the mortality of patients with HAI. The objective of this study was evaluate the prevalence of gram-negative organisms as causative agents of BSI in hospitals of São Paulo

Methods: Blood culture samples were collected from patients with BSI suspected from 27 private hospitals in the state of São Paulo-Brazil, between January and December 2018. The blood samples were collected in standardized BD BACTEC aerobic and anaerobic bottles and incubated in BACTEC FX (BD) until five days to detect bacterial growing. All positive samples were spread in plates with blood-agar, chocolate-agar and MacConkey and incubated at 35°C. After 24 hour incubation, sample of colonies was obtained from the plate using a sterile loop to identification of species by Matrix Assisted Laser Desorption Ionizated-Time of Flight (MALDI-TOF). Antimicrobial susceptibility tests were performed using Vitek 2 automated method. The data were evaluated retrospectively to calculate the prevalence of each gram-negative organism.

Results: Among 135,363 blood cultures that were performed, 9949 (7.4%) were positive. Considering all positive blood cultures, gram-negative organisms were identified in 3072 (30.9%) samples. The predominant gram-negative agents identified were: 1026 (33.4%) Escherichia coli, 814 (26.5%) Klebsiella pneumoniae and 191 (6.2%)

Enterobacter spp. Carbapenem-resistance was more frequently observed in K. Pneumoniae, 366 (45%) isolates were resistant to at least one carbapenem agent.

Conclusion: Gram-negative organisms are frequent causative agents of BSI in hospitals. The most common isolated agent was E. Coli, followed by K. Pneumoniae, that was the agent more frequently associated with carbapenem-resistance in this type of infection.

A-442

Prevalence, Distribution of Mixed Positive and Hybrid Strains of Diarrheagenic Escherichia Coli in Zhejiang Province, China

F. Yu, C. Chen, X. Chen, Y. Chen. *Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Department of Clinical Laboratory, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: To understand the prevalence of Diarrheagenic *Escherichia coli* (DEC) and the distribution of mixed positive and hybrid strains in Zhejiang Province.

Methods: DEC identification was performed on fecal specimens collected from outpatients with acute diarrhea in five sentinel hospitals in Zhejiang Province in 2016. Five independent lactose-fermenting colonies (5 colonies were not mixed) were picked from the MAC plates and DNA was extracted separately. 11 virulence genes were screened by multiplex PCR to identify and classify DEC into EAEC, ETEC, EPEC, STEC and EIEC. Compared the detection of DEC in each colony from the same specimen and analyzed the distribution of mixed positive and hybrid strains. A strain carrying virulence genes from multiple pathovars is called hybrid strain.

Results: A total of 1,007 fecal specimens (5,035 specimens of a single colony) were identified for DEC virulence gene, of which 539 (53.5%) were negative in 5 colonies and 468 (46.5%) were positive in at least one colony. Among 468 specimens, 135, 51, 44, 149 and 89 were found DEC positive in 1, 2, 3, 4 and 5 colonies, respectively. Of the 89 specimens with 5 DEC positive colonies, only 44 samples (49.4%) had the same virulence genes. Of 468 positive specimens, 333 (71.1%) were single DEC type positive (EPEC 156, ETEC 79, EHEC 48, EAEC 45, EIEC 5), 108 (23.1%) were mixed DEC types positive (most commonly both EHEC and EPEC positive simultaneously), and 27 (5.8%) were single hybrid strains. A total of 81 hybrid strains were isolated, among which the most common types were EPEC/EAEC (22), ETEC/EPEC (17) and EHEC/EAEC (13).

Conclusion: Identification of DEC must pick up several colonies. The detection rate of DEC in patients with acute diarrhea in this area was high, and mixed positive was common. If the study needs further analysis of a strain's characteristics, several colonies must be picked up in identification of DEC.

A-443

Decreased Frequencies of Th17 and Tc17 Cells in Patients Infected with Avian Influenza A (H7N9) Virus

J. Bao, D. Cui, X. Wang, Q. Zou, Y. Chen. *Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Department of Clinical Laboratory, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: The outbreak of avian influenza A(H7N9) virus infection, with a high mortality rate, has caused concern worldwide. Although interleukin-17 (IL-17) -secreting $CD4^+$ T (Th)17 and $CD8^+$ T (Tc)17 cells have been proven to play crucial roles in influenza virus infection, the changes in and roles of Th17 and Tc17 cells in immune responses to H7N9 infection remain controversial.

Methods: The frequencies of Th17 and Tc17 cells and IL-17A levels in samples were measured for 20 patients with confirmed H7N9 virus infection. 20 H1N1 patients and 20 healthy volunteers were selected as controls. For in vitro infection experiments, serum and PBMCs were isolated from 50 healthy volunteers and infected with H1N1 and H7N9 virus.

Results: We found that the frequencies of Th17 and Tc17 cells among human peripheral blood mononuclear cells (PBMCs) as well as IL-17A protein and mRNA levels were markedly decreased in patients with acute H7N9 virus infection. A positive correlation was found between the serum IL-17A level and the frequency of these two cell groups. In vitro infection experiments revealed decreased Th17 and Tc17 cell frequency and IL-17A levels at various time points post-infection. In addition, Th17 cells were the predominant sources of IL-17A in PBMCs of patients infected with H7N9 virus.

Conclusion: Taken together, our results indicate immune disorder in acute H7N9 infection, and a restored Th17 and Tc17 cell frequency might serve as a biomarker for predicting recovery in patients infected with this virus.

A-444

Performance Evaluation of the ADVIA Centaur and Atellica IM Zika Tests

K. Singh, J. Arroyo, M. Mansini, S. Patibandla. *Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

Background: Siemens Healthineers ADVIA Centaur® and Atellica® IM Zika Tests are fully-automated tests for the qualitative detection of IgM antibodies to Zika virus in serum and plasma. The ADVIA Centaur® and Atellica® IM Zika Tests each comprise two assays (Zika Ab and Zika IgM) and use an algorithm to determine the presence of anti-Zika virus IgM antibodies. Both assays are two-wash chemiluminometric immunoassays.

Methods: The performance of the ADVIA Centaur® and Atellica® IM Zika Tests were evaluated using serial draw samples obtained from Zika virus PCR-positive individuals, nonendemic samples collected from normal donors (U.S.) and pregnant women (U.S.), and endemic samples collected from symptomatic (Dominican Republic) and asymptomatic (Dominican Republic and Honduras) individuals. Performance of the ADVIA Centaur Zika Test on the ADVIA Centaur XP system was compared with performance of the Atellica IM Zika Test on the Atellica IM Analyzer. Cross-reactivity was evaluated with potentially cross-reactive samples. Precision and interference with common endogenous substances were assessed.

Results: The ADVIA Centaur® and Atellica® IM Zika Test algorithm was used on both ADVIA Centaur XP and Atellica IM analyzers. Each sample was first tested with Zika Ab assay, and samples with <0.8 index were reported nonreactive (negative) for Zika virus antibodies. The samples with ≥0.8 index were reflexed for testing with Zika IgM assay. The samples resulting <1.0 index with Zika IgM were reported as nonreactive (negative) for anti-Zika virus IgM antibodies. Samples showing ≥1.0 index (≥ two out of three replicates ≥1.0 index) with Zika IgM were reported as presumptive Zika-positive. The ADVIA Centaur Zika Test was reactive in serial draw samples (eight draws per individual) from all 36 Zika PCR-positive individuals tested on the ADVIA Centaur XP system 2-27 days after the appearance of symptoms. All serial draw samples collected between 8 and 14 days were reactive with the ADVIA Centaur Zika Test. The specificity of the ADVIA Centaur Zika Test was 99.89% (922/923) in the nonendemic population and 93.01% (173/186) in the endemic population. The ADVIA Centaur Zika Test demonstrated low cross-reactivity (1.47%; 5/341) and <15% interference with tested endogenous substances. In tested serial draw samples from 20 Zika PCR-positive individuals, positive percent agreement between the ADVIA Centaur XP Zika Test and Atellica IM Zika Test was 99.18% (121/122). On the Atellica IM Analyzer, the Zika Test was nonreactive in samples tested from all 353 nonendemic normal donors and pregnant women and demonstrated 100% agreement with ADVIA Centaur XP system results. Both the Zika Ab and Zika IgM assays demonstrated good precision (repeatability and within lab <10% CV) on the ADVIA Centaur XP and Atellica IM analyzers.

Conclusion: These results demonstrate good concordant performance of the Zika Test on ADVIA Centaur XP and Atellica IM analyzers.

A-445

Analytical Performance Characteristics of the New Beckman Coulter Access PCT Immunoassay*

K. Ley[1], K. Paulsen[1], G. Demirdjian[2], E. Cicirelli[1], I. Vigdorovich[1], J. Patzlaff[1], E. Ghimire[1]. [1]*Beckman Coulter LLC, Chaska, MN,* [2]*Beckman Coulter LLC, Marseille, France*

Background: Beckman Coulter has developed a highly sensitive procalcitonin (PCT) immunoassay for use on the Access Immunoassay Systems. PCT is a peptide of 116 amino acids with a molecular weight of ~13 kDa. PCT is produced in thyroid C-cells where it is converted to calcitonin in healthy individuals with less than 0.1 ng/mL PCT normally in circulation. PCT is a useful biomarker for diagnosis of sepsis and systemic inflammation because PCT levels increase in response to bacterial endotoxins and inflammatory cytokines.

Methods: The Access PCT assay is a sequential two-step sandwich assay. Rat monoclonal anti-PCT antibody alkaline phosphatase conjugate is added with sample to a reaction vessel and incubated. Paramagnetic particles coated with a mouse monoclonal anti-PCT antibody are then added and incubated. After washing, a chemiluminescent

substrate is added and light is generated which is directly proportional to the PCT concentration in the sample. The assay time to first result is ~20 minutes.

Results: The Access PCT assay demonstrates acceptable linearity with an analytical measuring range of 0.01 to approximately 100 ng/mL, with capability of measuring samples up to ~1000 ng/mL using automated dilution. In one study, based on CLSI EP17-A2, the Access PCT assay exhibited a Limit of Blank of 0.001 ng/mL, and using a minimum of 9 serum samples exhibited a Limit of Detection of 0.002 ng/mL and Limit of Quantitation of 0.002 ng/mL. A precision study was performed using seven serum samples tested using two replicates twice per day for 20 days. The total imprecision for serum samples with mean PCT concentrations from 0.090 to 76.31 ng/mL, resulted in %CV values of 3.8 to 7.2. Method Comparison with 229 patient samples using the Access PCT assay and the VIDAS® B·R·A·H·M·S PCT™ assay gave a Passing-Bablok Slope of 0.96 and Intercept of 0.08 ng/mL. The Pearson correlation coefficient was 0.99. Concordance analysis shows excellent agreement between Access PCT and three commercially available PCT assays (bioMérieux VIDAS B·R·A·H·M·S PCT, Abbott ARCHITECT B·R·A·H·M·S PCT and Roche Elecsys® B·R·A·H·M·S PCT), ranging from 95.8% to 97.3% at the 0.5 ng/mL cutoff and 92.7% to 99.2% at the 2.0 ng/mL cutoff.

Conclusion: The Access PCT assay is a highly sensitive and precise assay, demonstrating strong correlation and concordance to a well-established predicate PCT method at clinically relevant cutoffs.

*CE Marked. Pending clearance by the United States Food and Drug Administration; not available for *in vitro* diagnostic use in the U.S. Not for distribution in the United States.

A-446

An Evaluation of Performance of the VITROS®Immunodiagnostic Products HTLV I/II Assay*

P. Contestable, C. Noeson, R. Fazio, S. Clark, B. Novick, L. Sprague. *Ortho Clinical Diagnostics, Rochester, NY*

Background: This study was designed to assess the performance of the VITROS Immunodiagnostic Products HTLV I/II assay (VITROS HTLV I/II) on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600/XT7600 Integrated Systems. The assay detects human antibodies to human T-cell lymphotropic virus types I and/or II (HTLV I and HTLV II) in human serum or plasma.

Methods: Antibody detection in the VITROS HTLV I/II assay is achieved using four recombinant antigens (HTLV I and II envelope and HTLV I and II core antigens) coated onto the well. Sample is added to the coated wells in the first stage of the reaction, and HTLV antibody from the sample is captured. After washing, the four recombinant antigens conjugated to HRP are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent.

Specificity was assessed using 1000 random blood donor samples, including 500 serum and 500 plasma samples. Sensitivity was evaluated using 97 known HTLV positive samples, including 50 HTLV I and 47 HTLV II. Assay reproducibility was assessed using two reagent lots with a 9 member panel run in duplicate, two runs per day on 22 occasions covering 63 calendar days. A calibration interval of 63 calendar days was also evaluated.

Results: The specificity of the VITROS HTLV I/II assay for the blood donor population was 100.0% (1000/1000) [95% exact CI (99.63-100.00%)]. The sensitivity for known positive samples was 100.0% (97/97) [exact 95% CI (96.3-100.0%)]. For the reproducibility study the observed within-lab precision for the 7 reactive panel members ranged from 4.0 to 6.5 %CV on one reagent lot and 3.5 to 6.3 for the other reagent lot over 22 observations covering 63 calendar days. A calibration interval of 63 days was demonstrated.

Conclusion: The VITROS HTLV I/II assay is under development for use on the random access VITROS® Systems and has demonstrated 100% sensitivity and 100% specificity in the detection of HTLV I and II antibodies in human serum and plasma with excellent precision over a 63 day calibration interval. *Under development

A-447

Clinical Validation of the 510(k) Cleared-CLIA Waived Acucy™ Influenza A&B Test and Acucy™ Reader Using a Novel Composite Reference Method

S. Okuyama[1], K. Azuma[2], A. Hou[1], D. Schmidt[3], R. Herrmann[4], A. Grkovich[1], E. Gonzalez[1], D. Quan[1], H. Kuebitz[1], J. White[1], K. Kohno[2], S. Ward[1], S. Harris[1], H. Chan[1], M. Morita[2], S. Bradley[1]. *[1]Sekisui Diagnostics, San Diego, CA, [2]Sekisui Medical Co., Ltd., Ibaraki, Japan, [3]Sekisui Diagnostics, Lexington, MA, [4]Sekisui Medical Co., Ltd, Tokyo, Japan*

Background: The Acucy™ Influenza A&B Test with the Acucy™ Reader is the first rapid influenza test to evaluate performance against a novel composite reference method. The Acucy Influenza A&B Test is a qualitative lateral flow assay that detects and differentiates influenza A and influenza B viral antigens in nasopharyngeal and nasal swabs from patients with signs and symptoms of influenza using an Acucy test cassette. The test cassettes are analyzed by the Acucy Reader. In 2017, the FDA reclassified rapid influenza diagnostic tests from Class I to Class II and allowed clinical performance criteria requirements to be determined using either of two FDA accepted comparator methods, culture or molecular. This has allowed manufacturers to take a deeper look into the clinical performance of the test. The challenge with using a single method for reference testing is that each comparator method has its own inherent variation, making it challenging to understand performance of a newly developed test when compared separately to each method.

Methods: A prospective, multi-center study was conducted during the 2017-2018 influenza season to establish the performance and precision of the Acucy Influenza A&B Test and Reader. A total of 1003 prospective samples were included in the data analysis. The Acucy Influenza A&B Test was compared to a composite reference method, consisting of the consensus result from two FDA cleared molecular influenza A&B assays (Alere i Influenza A&B test and the Prodesse ProFlu+ test) and viral culture.

Results: The Clinical performance for the Acucy Influenza A&B Test is shown in the 2x2 tables, as percent sensitivity and specificity agreement to the composite reference method. The combined data for nasal and nasopharyngeal swabs for Influenza A detection had a sensitivity of 96.4% (95% CI: 93.1% - 98.2%) and a specificity of 96.0% (95% CI: 94.4% - 97.2%). The agreement to the composite reference method for the combined nasal and nasopharyngeal swab data for Influenza B detection had a sensitivity of 82.3% (95% CI: 75.6% - 87.4%) and a specificity of 98.1% (95% CI: 96.9% - 98.8%). Data tables show the precision of the Acucy Influenza A&B Test presented as percent agreement, average signal, and percent CV for within-laboratory repeatability, instrument-to-instrument reproducibility, lot-to-lot reproducibility, and user-to-user reproducibility.

Conclusion: Supporting studies from scientific literature indicate reference method anomalies can be normalized through composite reference analysis. Combining multiple tests to define a target disease status rather than using a single test is a transparent and reproducible method for dealing with the common problem of imperfect reference standard bias. To reduce this potential variability and gain a better picture of true clinical performance, a robust composite reference method was utilized to challenge the Acucy Influenza A&B Test across a range of FDA accepted comparators such as the two molecular platforms (Isothermal and PCR) and culture. Sekisui Diagnostics has used a novel composite reference method for the clinical validation of the Acucy Influenza A&B Test and Reader resulting in the 510(k) clearance-CLIA waived designation from the FDA in December 2018 and is now available for commercial sales in the US.

A-448

Results from Comprehensive Sexually-Transmitted Disease Testing in Men Utilizing a Consumer-Initiated, At-Home Kit

M. S. Francis, W. McAndrew, T. C. Pappas. *EverlyWell Inc, Austin, TX*

Introduction: Consumer-initiated, at-home sexually-transmitted disease (STD) testing may increase testing access where clinical services are limited, and also by decreasing the stigma of in-person testing. Prior publications have shown that men utilize health services far less than women, and providers are less likely to discuss sexual history and risk of STDs with men. This study examined rates of positive STD results in men utilizing an at-home STD kit over a twelve month period.

Methods: De-identified data from men who took a Men's STD Panel Test from September 2017 through September 2018 were obtained from EverlyWell's commercial database. All data were taken from commercial testing and there were no additional exclusion criteria other than age (18 yr or older) and gender, which were self-identi-

fied. The panels utilized liquid urine and dried blood spot as sample materials. A total of 1,779 test panels were completed from 1,697 unique individuals.

Results: All tests were run at an independent CLIA-certified laboratory and were validated at a minimum sensitivity and specificity for all analytes of 92% and 98%, respectively. Age range of men in this data set was 18 to 78. Overall, 12.1% of men received at least one positive STD result and 1% received more than one positive result on a single test panel. The most common STD resulted was Herpes Simplex Virus Type 2 at 9% followed by *Chlamydia trachomatis* at 2.6%. The largest age group utilizing this platform for testing was 30 to 34 years with 23% of total tests taken. The highest proportion of positives within a single age group was found in 65 to 69 years, 32% of the total men in that group, followed by 55 to 59 years, 28%. The highest absolute number of positive results was found in 25 to 29 years, 38/396 (10%) with half being positive for *C. trachomatis*.

Conclusions: At-home STD testing may allow men who would otherwise potentially be under-screened for a variety of reasons to easily manage their sexual health testing. Despite 65 to 69-year old men compromising only 1% of the total test takers in this data set, 32% of men in that cohort received a positive STD result. STD testing at home can easily be utilized by men of all ages to identify infections between routine health visits.

A-449

Development of Material for Use in a Proficiency Testing Programme for Procalcitonin

G. J. Davies, M. A. Thomas. *Cardiff and Vale UHB, Cardiff, United Kingdom*

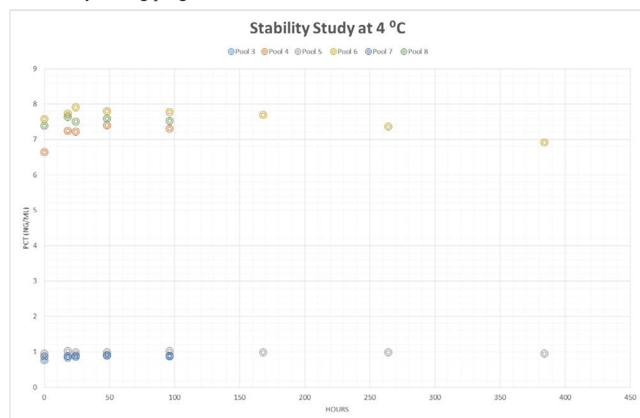
IntroductionProcalcitonin (PCT) is used as an infection biomarker and is used to improve the diagnostic work-up of patients with bacterial infections and its influence on decisions regarding antibiotic therapy.

Aim To develop and validate material for use in a Proficiency testing programme for Procalcitonin (PCT), including material stability and homogeneity.

Method Serum was spiked with recombinant PCT to concentrations of 1 ng/mL and 10 ng/mL and assessed for stability at 20°C and 4°C. The material was assayed using the Roche Cobas e601 and the Radiometer AQT90 PCT methods over 21 days at 20°C and 4°C with and without protease inhibitors. Three different protease inhibitors were assessed. The AQT90 was compared with the Roche Cobas assay using a series of samples ranging from 0 - 50 ng/mL. Within batch precision was assessed using 2 pools targeted at 1 and 10 ng/mL and between batch precision was assessed using the manufacturer IQC (low and high).

Results & Discussion Material without protease inhibitors showed a decay in PCT concentration of 13% at 1 ng/mL and 16% at 10 ng/mL within 24 hours at ambient temperature. Good results were achieved with one of the protein inhibitors with no significant decrease in PCT for 2 days at ambient temperature and 7 days at 4°C. The Radiometer AQT90 showed good comparison to the Roche Cobas e601 assay with linear regression showing a proportional positive bias of 8.8% and a constant negative bias of 0.76 ng/mL. Within batch precision showed CV of 2.8% and 5.0% at 1 and 10 ng/mL respectively. Between batch precision showed CV of 8.0% and 6.9% at 0.5 and 9.1 ng/mL respectively.

Conclusion The material shows good stability and can be utilised as part of the Weqas Proficiency testing programme.



A-450

Procalcitonin's Performance in Diagnosing Pneumonia in Real-World Practice

N. Ayala-Lopez, M. Ghaedi, D. R. Peaper, R. Harb. *Yale University, New Haven, CT*

Background: Procalcitonin (PCT) rises early upon bacterial infection and has a long-half life, making it useful in the diagnosis of infections and antibiotic stewardship. We sought to determine the utility of PCT in diagnosing pneumonia (PNU) in our patient population at Yale New Haven Hospital during real-world clinical practice.

Methods: PCT results from patients in 2015 were reviewed retrospectively. Patients were eligible for inclusion if all of following criteria were met: PCT in 2015 and a lower respiratory tract culture, respiratory virus testing, chest x-ray, ***and*** white blood cell (WBC) count within 1 day of the PCT. Patients who opted out of research and those with mycobacterial infections and culture-positive infections of body sites other than urine and lower respiratory tract were excluded from further analysis.

Results: A total of 400 patients remained with 413 eligible PCT results across the ED (109), ICU (134), and inpatient (167) and outpatient (3) areas with a mean age of 66.2±18.1 SD. PCT was higher in patients with multiple pathogens reported on their respiratory cultures, DFA or PCR tests (mean±SD=2.43±0.74 ng/ml; N=32), than those with no pathogens reported (3.25±1.13 ng/ml; N=224; $p<0.05$). Patients were grouped for the presence or absence of clinically defined PNU, according to a modification of the Centers for Disease Control (CDC) PNU1 criteria incorporating: 1) chest x-ray results, 2) altered WBC number/altered mental status/fever, and 3) respiratory/breathing signs. PCT was higher in patients with clinically defined PNU, and the high PCTs were consistent with positive chest x-rays (criteria #1), and positive criteria #2, but not criteria #3. Incorporation of an elevated PCT >0.1 ng/mL into the PNU-score slightly improved the area under the ROC curve (AUC) for the algorithm's detection of PNU against the final clinical diagnosis (0.73 without PCT vs. 0.76 with PCT). Furthermore, higher PCT was associated with higher thirty-day and one-year mortality.

Conclusion: PCT results were largely consistent with other markers of PNU such as imaging and CDC criteria #2, which suggests that PCT can be useful in evaluating for the presence PNU. However, the PCT may not add additional information to assist in decision making above the already commonly ordered tests.

A-451

Prevalence of Methicillin-Resistant Staphylococcus Aureus (MRSA) in Wound Culture from Residents in Long-Term Care Facilities

R. Khoury, P. Patel, A. Gandhi, H. Massey, D. Gudaitis, P. Gudaitis. *Aculabs, Inc., East Brunswick, NJ*

Background: Methicillin-resistant *Staphylococcus aureus (MRSA)* is a staph bacterium that is resistant to many antibiotics. It can cause a life threatening bloodstream infection, pneumonia and wound infections. Several studies have shown prevalence of infections caused by MRSA to be 5%-40% in Long-Term Care Facilities, where they can spread rapidly because most of the residents are elderly, frail and are on multiple medications in addition to other age-associated risk factors. Beyond the clinical health issues that MRSA causes, it also increases health costs for the patient and the facility.

Design: Data was collected from 3,000 wound culture samples from patients residing in long-term care facilities in 2018. All specimen were processed and results are considered negative when no growth of any pathogenic organism or growth of normal skin flora were seen. If any significant organisms were present after the incubation, identification and susceptibility tests were performed utilizing MicroScan Walkaway 96 conventional panels. For any culture where Staphylococcus aureus was identified and antimicrobial resistance to oxacillin has been demonstrated, the Final Report is flagged as MRSA**.** Statistical analysis was done using Analyse-it.

Results: 90.4% of the cultures were positive. MRSA was the most common bacteria (16.2% of all positive cultures and 61.9% of all drug resistant cultures) followed by proteus mirabilis, enterococcus faecalis, pseudomonas aeruginosa, and staph aureus coagulase negative. We also noticed a decrease in MRSA prevalence from previous years.

Conclusion: MRSA is still the most prevalent bacteria in wound infections and a major health care concern in long-term care facilities. Although the prevalence is going down, more work is needed. A successful prevention program requires combined effort from healthcare providers and facilities. The facilities should make MRSA infection a priority, implement antibiotic stewardship and infection control plan, identify the risk factors for the infection and evaluate their progress. Healthcare providers can help by following the current prevention recommendation for device and procedure related infections. They should also treat infections using the appropriate antibiotics if

they do occur; in addition to educating the patients and healthcare workers about ways to prevent infection and the spread to other residents.

A-452

Use of a Rapid Immunochromatographic Test for Diagnosis of Diarrhea Caused by Clostridioides Difficile

V. A. C. Castro-Lima, A. B. Mauro, N. F. Souza Jr., M. E. Araujo, L. F. Franco. *Diagnostico das Americas - DASA, São Paulo, Brazil*

Background: It has been observed an increase in incidence of diarrhea caused by Costridioides difficile in recent years worldwide. The main risk factors for this condition are previous antibiotic use, elderly people and long length stay in hospital. There are several current methods for diagnosis of C. difficile infection: enzymatic and immunochromatographic assays for detection of toxins and antigens, such as glutamate dehydrogenase, directly from feces samples. Biomolecular tests, that use polymerase chain reaction (PCR) for detection of genes associated with production of toxins, are being more used in recent years and have high sensitivity.

Methods: There were collected feces samples of patients admitted in hospitals of the city of Sao Paulo, Brazil, with diarrhea and suspicion of infection by C. difficile. The immunochromatographic rapid test (ICRT) C. difficile Quick Check Complete (TECHLAB, United States) was performed for all samples. To compare the results of the rapid test it was used an enzymatic or biomolecular assay: Remel - Xpect C. difficile Toxin A/B Test (Termo Scientific, United States) or PCR Xpert C. difficile (Cepheid, United States), respectively. Kappa index was calculated to evaluate the concordance among methods.

Results: Thirty-eight feces samples were evaluated using ICRT and enzymatic assay, whereas PCR were performed for 27 of them. Nineteen (50.0%) samples were positive for C. difficile by both ICRT and enzymatic assay, with a total concordance among methods (κ=1.0). There were five (18.5%) samples positive for C. difficile by PCR with two discordances comparing with ICRT: one sample was PCR-positive and ICRT-negative and the other was PCR-negative and ICRT-positive (κ=0.75).

Conclusion: In our study we have observed that ICRT presented a substantial concordance comparing PCR and an almost perfect concordance comparing with enzymatic assay, based on kappa index, for diagnosis of diarrhea caused by C. difficile. Considering that, using this method, the result can be obtained in a quick and easy way, allowing an early start of treatment and isolation measures, ICRT should be considered for use in routine lab for diagnosis of this type of infection.

A-453

Diagnosis of Pulmonary Tuberculosis through of Koch Bacillus Culture and BAAR Survey in the Last Five Years in the State of Pernambuco, Brazil

G. A. Campana[1], C. M. G. Pessoa[2], C. R. P. Soares[2], D. C. Pimentel[2], D. A. H. Medeiros[2], D. A. Silva[2], A. M. Neto[2], M. A. Rodrigues[2], P. S. R. Araújo[2], E. P. Falcão[2]. *[1]DASA, São Paulo, Brazil, [2]DASA, Recife, Brazil*

Background: Active tuberculosis (TB) is a global public health problem and one of the world's leading causes of mortality. In Brazil, in 2017, 69,569 new cases were reported. In the same year, the incidence coefficient was 33.5 cases/100 thousand inhabitants. The state of Pernambuco is the third in incidence rate with 46.0/100 thousand inhabitants and has the second highest mortality rate due to TB, 42,0/100 thousand inhabitants. Early detection and treatment of TB reduce the transmission of Mycobacterium tuberculosis complex. In areas with sufficient resources and high levels of experience with the range of clinical presentations of TB, approximately 20% to 30% of patients with TB are found to lack culture confirmation and are diagnose clinically. The diagnosis of pulmonary TB should be suspected in patients with relevant clinical manifestations (cough more then 2 to 3 weeks duration, lymphadenopathy, fevers, night sweats, weight loss) and relevant epidemiologic factors. Additional diagnostic tools include sputum acid-fast bacilli (AFB) smear and culture. The detection of AFB on microscopic examination of stained sputum smears is the most rapid and inexpensive TB diagnostic tool. Smears may be prepared directly from clinical specimens or from concentrated preparations. Sputum should be of good quality, may be obtained spontaneously (by coughing) or it may be induced, and at least 3 mL in volume. Sputum AFB smears are less sensitive than culture. To compare the frequency of positivity of sputum exams through culture of M. tuberculosis with the search for BAAR in the diagnosis of pulmonary tuberculosis in sputum samples from 2013 to 2018 in the State of Pernambuco. **Method:** Were evaluated 749 sputum samples from individuals with presumptive diagnosis of pulmonary tuberculosis through isolation using Lowenstein-Jensen's medium (JL) for culture of Koch's bacilli (CBK). And 10,570 sputum samples were collected for the study of alcohol-acid resistant bacilli (BAAR) by the method of staining of Neelsen ziehl for the detection of M. tuberculosis. **Results:** Of the 749 samples, submitted to culture bacteriological (5.3%) percent of the sputum specimens were positive, and of the 10.570 (3.2%) of the sputum samples were positive for smear microscopy. The mean age of the culture positive patients was 47.9 and the mean age of the patients for AFB screening was 43.27. Of these patients, the presence of M. tuberculosis in the microscopy sputum samples was 114 positive (+), 167 positive (++) and 57 positive (+++). The proportion of positivity in both tests compared was similar. However, the demand for Bacillus culture exams is still very low in relation to the smear exams requested by the medical team. Being the diagnostic microbiological laborious with protocol of up to 45 days, the methodology molecular tests have been shown to be quite promising in the diagnosis of TB in the detection of the pathogen and the resistance gene. **Conclusion:** In the scenario of developing countries the frequency of pulmonary TB is increased and deserves attention of public health in diagnostic and treatment. Therefore, the implantation of fast diagnostic and accurate would help the reduction in mortality rates.

A-454

Susceptibility Profile of Neisseria Gonorrhea in the Dominican Republic

A. J. Mena Lora[1], M. Thormann[2], G. Grau[3], P. Gonzalez[3], B. Billini[3], S. C. Bleasdale[1]. *[1]University of Illinois at Chicago, Chicago, IL, [2]Hospital Salvador B Gautier, Santo Domingo, Dominican Republic, [3]Laboratorios Amadita, Santo Domingo, Dominican Republic*

Background: *Neisseria gonorrhea* is one of the most common sexually transmitted infections (STIs) worldwide. Rising resistance rates to common antimicrobials against *Neisseria* is a global public health threat. We seek to describe the susceptibility patterns and geographic distribution of resistance for *Neisseria* in the Dominican Republic (DR).

Methods: This is a retrospective review of the phenotypic susceptibility profiles of *Neisseria gonorrhea* isolates from a clinical laboratory in the DR (Amadita Laboratories). Amadita provides services nationwide. Data corresponded to isolates from adult male urethral samples in the period of January-December 2018. Age, sex, antimicrobial susceptibility patterns, and the geographic distribution based on sample collection location was tabulated. Automated susceptibility testing (Vitek®2, bioMérieux) was used.

Results: A total of 128 positive cultures for *Neisseria gonorrhea* were reviewed. Samples were widely distributed in 22 locations corresponding to 8 cities. All patients were male and all samples were collected from urethral source. Ages ranged from 18 to 68, with an average of 28. Susceptibilities are reported in Table 1.

Table 1. Susceptibility profiles for *Neisseria gonorrhea*

n=128	Ciprofloxacin	Ceftriaxone	Tetracycline	Spectinomycin	Penicillin
Sensitive	20%	96%	51%	97%	10%
Intermediate	19%	0%	20%	0%	0%
Resistant	61%	4%	29%	3%	90%

Conclusion: Resistance to ceftriaxone and spectinomycin was low. The percentage of isolates with ciprofloxacin, tetracycline and penicillin resistance was high. These agents may not provide adequate empiric coverage. Though spectinomycin retains susceptibility, it no longer is recommended due to lack of efficacy in pharyngeal gonorrhea. Ceftriaxone is the optimal empiric choice for gonorrhea in the DR. Continued surveillance of antimicrobial susceptibilities for *Neisseria* is important to identify changes in resistance and ensure proper treatment recommendations.

A-455

A Rapid and Precise Molecular Detection of Aerobic Vaginitis Using the Hologic Fusion Open Access Platform

M. J. Beckman[1], S. M. Jacobs-Helber[1], A. Murphy-Colfax[1], J. C. Shultz[1], O. Girard[2]. *[1]GENETWORx, Glen Allen, VA, [2]Hologic, San Diego, CA*

Background: GENETWORx was founded in 2011 on the principle that the combination of laboratory testing and pharmacy based therapeutic recommendations provides a powerful aid to clinicians in pursuit of better patient outcomes. Aerobic Vaginitis represents a recent classification of vaginal infection with abnormal aerobic flora that are prone to immunoreactivity and antibiotic resistance. AV may represent a distinct subset of patients presenting with Bacterial Vaginosis but resistant to traditional ther-

apy. Additionally, AV if misdiagnosed for BV can persist despite treatment and lead to complication with pregnancy. In this study, we have designed a molecular test to detect AV in vaginal swab samples and have adapted this molecular test from a Real-Time PCR to be carried out on the Hologic Fusion as a fully automated test with bi-directional LIMS interface. The purpose of this study was to generate a rapid screen for bacterial testing and automated reflex to an antibiotic resistance. **Methods:** GENETWORx has developed a rapid molecular screen for aerobic vaginitis (AV) on the Hologic Panther Fusion designed with increased flexibility, improved workflow, and enhanced productivity, based on the pathogen and antibiotic resistance genes detected. Vaginal swab collections were transported to GENETWORx in a BD universal viral transport medium (UTM). Sample aliquots were transferred to LIM broth for overnight culture of symptomatic streptococcus agalactiae. Approximately 1 ml of culture was added to an Aptima Specimen Transfer tube. Aptima Specimen tubes labeled with barcoded case information for each patient were loaded into the Panther Fusion for a single extraction. Test combinations were added as Open Array Multiplex Assays for (1) infectious bacteria; *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Enterococcus faecalis*, and (2) antibiotic resistance factors; *ermA*, *ermB*, and *ermC*. Cases that are positive for bacteria auto-reflex through an on-board Bi-directional laboratory information system (LIS) triggering the antibiotic resistance assay to determine for the presence or absence of *erm* resistance factors. **Results:** A single extraction of one sample can be run with the combination of two separate Panther Fusion AV tests. Select testing from the most common AV infectious organisms allows for running only the needed assays to avoid unnecessary expenses associated with expanded panels for bacterial vaginosis testing to more precisely distinguish AV infections. The inclusivity of the Open Array multiplex format and reflex of positive infections to a similar multiplex antibiotic resistance assay allowed for both high-throughput testing and rapid resulting to patient data directly into the LIS interface. Also, the bi-directional interface ability of the Panther Fusion system, allows cases to be entered remotely.

Conclusion: Bacterial vaginosis (BV) is treated with traditional metronidazole therapy that targets anaerobic bacteria, and often a subset of approximately 10-20% of these patients fail to respond to therapy and this can lead to complications of pregnancy such as premature rupture of membranes and preterm delivery. This study provides a documented rapid molecular testing platform for aerobic vaginitis utilizing the Hologic Panther Fusion system. Use of this test will reduce user interaction and return visits to the workflow system for increased resulting and increased lab efficiency.

A-456

Development of Dengue Panels for Serology and Molecular Assay Validation

E. Morreale, J. Leach, C. Huang, J. Wu, B. Anekella. *SeraCare, Milford, MA*

Background: Dengue fever, which is caused by four different serotypes of dengue viruses, is a continuing threat for half of the global population. Epidemics caused by this illness are increasing in geographical areas such as Asia and Latin America. Each year it is estimated that close to 400 million people will come in contact with one of the four dengue viruses and 25% of those exposed will experience clinical symptoms. Clinicians depend on rapid and accurate diagnostics to dictate appropriate treatment modalities for those infected with dengue and other similar flaviviruses. Molecular and serological assays for dengue diagnostics have both strengths and weaknesses based on their technology. Serological assays while cost-effective and easy to run can cross-react with similar flavivirus antibodies, leading to false positive results. Molecular assays are challenging to run in areas with limited resources and infrastructure but have much greater specificity and sensitivity over serological assays. In both cases, well-characterized control specimens are needed for assay developers to create more robust assays that are specific to dengue detection.

Methods: SeraCare has developed two panels to meet these needs; the AccuSet™ Dengue Performance Panel and the AccuTrak™ Dengue Synthetic RNA Qualification Panel.

The AccuSet Dengue Performance Panel is a 15-member performance panel consisting of undiluted, naturally occurring plasma samples from dengue endemic geographic regions with reactivities ranging from negative to positive for Dengue virus RNA, NS1 antigen, and IgM/IgG antibodies. The AccuTrak™ Dengue Synthetic RNA Qualification Panel is a 4-member panel consisting of synthetic RNA sequences from Dengue virus 1 strain US/Hawaii/1944, Dengue virus 2 strain New Guinea C, Dengue virus 3 strain H87 and Dengue virus 4 strain H241. Each serotype genome was manufactured from transcribed RNA's representing the entire genomic RNA that were quantitated to ~50,000 copies/mL using digital droplet PCR. Testing of both dengue panels was performed on commercially available and laboratory-developed assays for serological and molecular detection of the dengue virus.

Results: Comparative analysis between dengue assays demonstrated agreement across manufacturers and technologies. The AccuTrak Dengue Synthetic RNA Qualification Panel was tested on an LDT molecular assay with primers and probes similar to the CDC DENV 1-4 RT-PCR assay along with reproducibility testing on the Altona RealStar® Dengue RT-PCR Kit 2.0 and molecular LDT assays for dengue at ARUP Laboratories and Quest Diagnostics. Both panels provide well-characterized control specimens for researchers developing or validating molecular and/or serology-based dengue assays.

Conclusions: SeraCare has developed a suite of dengue panels for molecular and serological test methods that will enable researchers and diagnostic manufacturers to expedite the development, evaluation, validation and verification of molecular and serological dengue assays. Due to the cross-reactivity and specificity issues of serological assays alone, it is advised for clinicians to follow a proper diagnostic algorithm that encompasses both molecular and serological dengue test methods, if available, along with routine patient monitoring. A proper clinical management program with state and local vector control and surveillance programs will ensure preparedness for future outbreaks of this disease.

A-457

Performance Evaluation of ADVIA Centaur Type-Specific Herpes-1 IgG and Herpes-2 IgG Assays

T. Marti. *BioKit, Barcelona, Spain*

Background: Two members of the herpes virus family, Herpes Simplex Virus 1 and 2 (HSV-1 and HSV-2) are pervasive and infect epithelial cells. HSV-1 is normally associated with oral infections and HSV-2 is associated with genital infections.[1] ADVIA Centaur® Herpes-1 IgG and ADVIA Centaur Herpes-2 IgG are indirect sandwich chemiluminescent two-step immunoassays, which use the recombinant HSV-1 and HSV-2 glycoprotein G (gG1 and gG2), respectively. The assays are designed to detect HSV-1 IgG and HSV-2 IgG type-specific antibodies to quickly recognize herpes simplex virus infection for early initiation of therapy. The aim of this study was to evaluate the performance of both assays on ADVIA Centaur systems.

Material/Methods: Sensitivity and specificity for the ADVIA Centaur Herpes-1 IgG and ADVIA Centaur Herpes-2 IgG were tested in a clinical study with serum samples from 864 adults (≥ 18 years of age), which included 274 pregnant women, submitted for HSV routine testing against a commercially available anti-HSV-1 IgG and anti-HSV-2 IgG immunoblot method and a Western Blot reference confirmatory test (University of Washington, Seattle). Other relevant performance characteristics like the analysis of a Centres for Disease Control CDC panel, 20 day precision (CLSI EP5-A3), reagent open on-board stabilities (CLSI 25-A), interferences (CLSI EP7-A2) and precision. Interferences and cross-reactivity were also determined.

Results: ADVIA Centaur Herpes-1 IgG sensitivity was 97.5% (507/520) and specificity was 96.2% (331/344), respectively. ADVIA Centaur Herpes-2 IgG sensitivity was 95.3% (245/257) and specificity was 98.5% (598/607), respectively. Both assays showed a 100% agreement with CDC herpes panel results. Repeatability and within-lab %CV was found to be between 3.1%-5.0% and 4.5%-7.8% respectively for ADVIA Centaur Herpes-1 IgG, and between 1.7%-4.3% and 5.9%-7.6% respectively for ADVIA Centaur Herpes-2 IgG. Reagent open on board stability was verified up to 60 days for both assays. Both assays show no interferences for Biotin, Hemoglobin, Hyperproteinemia, Hypoproteinemia, Complex and Free Bilirubin, Triglycerides and Cholesterol, or cross-reactivity to 30 different disease conditions, which include other viral antibodies, disease-state specimens, and populations.[2]

Conclusions: The results of the performance studies show that the ADVIA Centaur Herpes-1 IgG and ADVIA Centaur Herpes-2 IgG assays have good precision and sensitivity that can determine type-specific HSV-1 IgG and HSV-2 IgG antibodies qualitatively in human serum or plasma (EDTA and lithium heparin). Both tests are indicated for testing sexually active adults or expectant mothers to aid in the presumptive diagnosis of HSV-1 or HSV-2 infection.

The performance of two type-specific herpes assays together with random access, easy-to use, and full automation ADVIA Centaur systems make them a good choice for routine use in a hospital or clinical laboratory.

Footnotes:

[1] https://www.who.int/news-room/fact-sheets/detail/herpes-simplex-virus

[2] IFU References: ADVIA Centaur XP/XPT Herpes-1 IgG IFU = 10720846XP_EN Rev. A, 2019-02, ADVIA Centaur XP/XPT Herpes-2 IgG IFU = 10720849XP_EN Rev. A, 2019-02

A-458

Performance Evaluation of the ADVIA Centaur EBV-VCA IgM, EBV-VCA IgG, and EBV-EBNA IgG Assays

T. Marti. *BioKit, Barcelona, Spain*

Background: Epstein-Barr virus (EBV) is one of the most common viruses in humans. Most individuals become infected during childhood, and it is estimated that seroprevalence in adults is close to 95%.[1] EBV is the causative agent of infectious mononucleosis (IM) and is associated with other diseases such as Burkitt's lymphoma and nasopharyngeal carcinoma.[2] Using—viral capsid antigen (VCA) IgM, VCA IgG, and EBV nuclear antigen (EBNA) IgG—it is possible to distinguish acute from past infection.[3] The ADVIA Centaur® EBV-VCA IgM, EBV-VCA IgG, and EBV-EBNA IgG assays are indirect sandwich two-step immunoassays that use chemiluminescent technology. They are designed for the qualitative detection of VCA IgM, VCA IgG, and EBNA IgG antibodies in human pediatric and adult serum and plasma (EDTA and lithium heparin) samples. The aim of this study was to evaluate the performance on the ADVIA Centaur XP System.

Material/Methods: Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the ADVIA Centaur EBV-VCA IgM assay were assessed by testing 789 serum samples against the DiaSorin LIAISON EBV IgM assay. For ADVIA Centaur EBV-VCA IgG, 886 serum samples were analyzed against the DiaSorin LIAISON EBV VCA IgG assay. The VCA IgM and IgG samples were provided by different commercial sources. For the ADVIA Centaur EBV-EBNA IgG assay, 1481 serum and 279 plasma samples were evaluated against the DiaSorin LIAISON EBV EBNA IgG assay. Performance characteristics including precision, reagent onboard stability, interferences, and cross-reactivity with other disease conditions were also evaluated across all three analytes.

Results: Evaluation of patient samples using the ADVIA Centaur EBV-VCA IgM assay indicated a PPA% of 96.6% and a NPA% of 96.1% compared to the DiaSorin LIAISON EBV IgM assay results. The assay demonstrated good preliminary precision, with repeatability and within-laboratory %CV of 2.1-10.9% and 2.7-4.7% respectively. For the ADVIA Centaur EBV-VCA IgG assay, PPA% was 99.5% and NPA% was 97.8%. Repeatability and within-laboratory %CV were 1.9-4.5% and 3.5-10.4% respectively. For the ADVIA Centaur EBV-EBNA IgG assay, PPA% was 98.7% and NPA% was 97.2%. Repeatability and within-laboratory %CV were 2.1-3.0% and 3.1-4.8% respectively. Reagent onboard stability was verified up to 8 weeks, and no significant endogenous interferences (biotin, hemoglobin, hyperproteinemia, hypoproteinemia, bilirubin (complex and free), triglycerides, and cholesterol) or significant cross-reactivity (≤ 10% change in results) with 22 different disease conditions were observed for the three assays.

Conclusions: Study results demonstrate good preliminary performance of the prototype ADVIA Centaur EBV assays.

* Under development. Not available for sale. Performance characteristics of these products have not been established. Future Availability not guaranteed.

Footnotes:

[1 21, 2] E. Sickinger, et al. Comparative evaluation of the new ARCHITECT EBV assays considering different testing algorithms. *Diagnostic Microbiology and Infectious Disease* (2014).

[3] M. De Paschale et a;. Serological diagnosis of EBV infection: Problems and solutions. *World Journal of Virology* (2012).

HOOD05162002980203

A-459

Comparison of QuantiFERON-TB Gold In-Tube and QuantiFERON-TB Gold Plus in Screening of Latent Tuberculosis on Healthcare Workers

S. Kang, W. Lee, M. Kim. *KyungHee University School of Medicine, Seoul, Korea, Republic of*

Background: Health care workers (HCWs) are at risk of infecting tuberculosis (TB), especially in countries with high TB incidence. Therefore, annual latent TB screening using TST and interferon gamma release assay (IGRA) on HCWs is performed. However, IGRA, which is mainly used for screening, shows considerable variability due to several pre-analytical, analytical and patient related factors. The aim of this study was to compare the performance of the QuantiFERON-TB Gold Plus (QFT-Plus; Qiagen, Germantown, MD) with the QFT-Gold In-Tube (QFT-GIT; Qiagen) on health care workers, especially in agreement of dichotomous results and the proportion of subjects in borderline range (0.20-0.99 IU/mL).

Methods: Annual latent TB screening on HCWs is performed in KyungHee University Hospital at Gangdong and 105 HCWs were included in this study. Whole blood were collected directly into the 3 QFT-GIT and 4 QFT-Plus tubes and immediately transported to the laboratory. The QFT-GIT and QFT-Plus assays were performed according to the manufacturer's instructions. Measurements of INF-γ were performed on the Agility (Dynex Technologies, Chantilly, VA) automated ELISA analyzer. The same interpretation criteria apply to both assays but QFT-Plus results are considered positive if either one or both of the TB antigen tubes are positive.

Results: The agreement of QFT-Plus with QFT-GIT was 99/100 (99%) negative tests and 3/5 (60%) positive tests and showed overall agreement 102/105 (97.1%) with a Cohen's kappa value of 0.652 (Good agreement). There were 3 discordant subjects and their INF-γ values (TB antigen tube-Nil tube) of QFT-Plus TB1, QFT-Plus TB2 and QFT-GIT were as following: Subject 1) 0.1, 0.08, and **1.121**, Subject 2) 0.22, 0.21 and **0.689**, Subject 3) 0.02, **0.38**, and 0.017. Subjects with borderline range were 7 in QFT-GIT and 9 in QFT-Plus and there were 6 discordant subjects.

Conclusion: According to manufacturer, the QFT-Plus is more sensitive than QFT-GIT for detecting both latent and active TB infection. But QFT-GIT had higher positive rate than QFT-Plus although there was high overall agreement (97.1%) between QFT-GIT and QFT-Plus. In addition, QFT-Plus had more subjects with a borderline range than QFT-GIT and there was considerable discrepancies in subjects with a borderline range between QFT-GIT and QFT-Plus, even though two assays were performed with simultaneously collected specimens.

Wednesday, August 7, 2019

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

Lipoproteins and Vascular Diseases

B-001

Does Dyslipidaemia Independently Predict Elevated Carotid Intima Media Thickness in Nigerian HIV-Infected Individuals?

L. C. IMOH, C. C. Ani, K. O. Iyua, A. I. Odo, G. A. Amusa, G. O. Osaigbovo, C. U. Uhunmwangho, Z. M. Gimba, E. E. Abene, C. O. Isichei, B. N. Okeahialam. *JOS UNIVERSITY TEACHING HOSPITAL, JOS, Nigeria*

Background: HIV is fast gaining reputation as a leading cause of cardiovascular diseases (CVDs) in sub-Saharan Africa. The burden of HIV remains high in sub-Saharan Africa (SSA) and Nigeria is among the countries with the highest prevalence. Carotid Intima Media Thickness (CIMT) is a non-invasive marker for arterial stiffness and atherosclerosis and a predictor for CVDs. The significance of traditional risk factors of CVDs in HIV-infected population is still a subject of research. We examined the association between traditional risk factors for CVDs and CIMT in Nigerian HIV-infected individuals.

Method: This was a cross-sectional study carried out at the AIDS Prevention Initiative of Nigeria (APIN)-supported HIV clinics at Jos University Teaching Hospital and Faith Alive Foundation in Jos, Nigeria. Randomly selected HIV patients with undetectable viral load were recruited into the study.

Socio-demographic data, cardiovascular risks factors, relevant medical history and information regarding HIV related factors were obtained from patients' records. Biophysical and anthropometric measurement, Carotid Intima Media Thickness, and relevant laboratory parameters (fasting lipid profile, fasting plasma glucose) were also assessed. Impaired Fasting glucose was defined as fasting glucose ≥ 6.1mmol/L and dyslipidemia was defined as one or more of fasting TC ≥5.2mmol/L, HDL-C <1.0mmol/l, TG ≥ 1.7mmol/L or LDL-C ≥ 3.4mmol/L according to NCEP guidelines. Data were analyzed with the Statistical Package for Social Sciences (SPSS® Incorporated Chicago Version 23.0) software. Bivariate analysis (chi square test) and multivariate logistic regression were used to examine the relationship between risk factors of CVD and elevated CIMT. The significance level was set at p≤ 0.05.

Results: One hundred and fifty HIV-infected participants were enrolled into the study with a mean age of 42.0 (9.4) years. One hundred and five (70%) were women. The mean and median (IQR) CIMT were 0.59mm and 0.58 (0.52-0.65) mm respectively. Elevated CIMT was regarded as CIMT≥0.77mm which corresponded to the 90th percentile of CIMT.

Overall, dyslipidemia was the most common traditional risk factor for CVD observed in Nigerian HIV-infected individuals (46.0%), followed by Hypertension, (26.0%). Male gender, Age > 40yrs, increased systolic blood pressure (SBP) ≥ 140mmhg were significantly associated with elevated

CIMT, (P= 0.015, 0.001, 0.003) respectively. There was however no association between increased diastolic blood pressure (DBP) ≥ 90mmhg, abnormal lipid fractions (TC, HDL, TG and LDL), impaired fasting glucose, obesity, Family history of DM, hypertension or stroke with increased CIMT (P> 0.05). Following a multivariate analysis using binary logistic regression, only elevated SBP remained as independent predictor of elevated CIMT [Adjusted OR = 5.2 (1.2-22.8.)] after controlling for covariates such as: sex, age, DPB, dyslipidemia, glycemic status and obesity.

Conclusion: Although the prevalence of dyslipidemia was high, it did not predict carotid arterial stiffness (marked by elevated CIMT) in Nigerian HIV-infected individuals. However, increased SBP was the major factor independently associated with elevated CIMT in this population.

B-002

Assessing the Correlation Between Serum Lipids and Glucose Values and Sensorineural Hearing Loss Among Patients Attending ENT Clinic at Calabar Teaching Hospital

L. EKPE. *university of calabar teaching hospital, CALABAR, Nigeria*

Background: Sensorineural hearing loss (SSHL) is a common hearing defect among elderly patients. The link between high serum lipids and SSHL has been a subject of concern and research for many years. This study aimed to describe the pattern of lipid abnormalities in patients with SSHL and consider if there is a correlation between these values and SSHL.

Method: This cross sectional study recruited 40 patients attending the ENT clinic of University of Calabar Teaching Hospital between May 2017 and October 2018 after obtaining ethical clearance. Recruited patients were aged between 20-65years with average age of 50.2 years and a peak incidence of SSHL at fifth decade. Of the total,23 were males(57.5%) and 17 were females(42.5%) and a matched control of subjects aged 30-60 years with mean age of 50years consisting 20 males(40%) and 30 females(60%).In both groups, fasting serum lipids {(Total cholesterol(TC), high density lipoprotein cholesterol(HDL),and Triglycerides(TG),low density lipoprotein cholesterol(LDL)} and fasting plasma glucose were assessed.

Results: Statistical evaluation of the parameters in both patients and controls did not yield any statistical difference. This implies that in this study, SSHL is not directly linked to levels of lipids.

Conclusion: Abnormal lipid values have no correlation with SSHL.

B-003

miRNA Predictive Profile Based on <*LDLR*>, <*APOB*> and <*PCSK9*> 3'UTR Variants as Potential Biomarker for Familial Hypercholesterolemia

R. C. C. Freitas[1], R. H. Bortolin[1], J. B. Borges[2], B. Los[1], C. D. Hernandez[1], A. A. Mori[1], V. F. Oliveira[1], G. M. Ferreira[1], E. S. R. Marçal[1], G. M. Bastos[2], C. M. Fajardo[1], R. M. Gonçalves[2], A. A. Faludi[2], A. D. Luchessi[3], R. D. C. Hirata[1], M. H. Hirata[1]. *[1]University of Sao Paulo, Sao Paulo, Brazil, [2]Dante Pazzanese Institute of Cardiology, Sao Paulo, Brazil, [3]Federal University of Rio Grande do Norte, Natal, Brazil*

Background: MicroRNAs (miRs) have emerged as a potential tool to understand the pathophysiological mechanisms in diseases not fully explained by genetic factors, such as Familial Hypercholesterolemia (FH). This study identified *LDLR*, *APOB,* and *PCSK9* variants within the 3' untranslated region (3'UTR), which are binding sites for miRs, and evaluated *in silico*. **Methods:** DNA from 48 patients with FH (according to the Dutch MEDPED criteria) was sequenced using the Illumina MiSeq System platform. MirSNP online tool was used to predict the effect of functional 3'UTR variants on miRs profile. **Results:** Functional and/or pathogenic exome variants in *LDLR*, *APOB* and *PCSK9* were identified in 26 patients with molecular diagnosis of FH (MD group). In *LDLR*, rs10409044, rs17243011, rs200039283, rs10415069 and rs28398082 3'UTR variants were found only in MD group, while rs3180023, rs17243018, rs142697277 and rs72658874 in non-MD group. *LDLR* rs5742911, rs17242683 and rs55971831 variants were more frequent in MD group and rs3826810 and rs7254521 had higher frequency in non-MD group (p<0.05). For *APOB* rs12720763 and rs72654428 3'UTR variants were identified in one patient from non-MD group. In *PCSK9*, rs149837083 3'UTR variant was detected only in MD group, and rs17111557 was more frequent in MD than in non-MD group. No *PCSK9* 3'UTR variant were found only/and or most frequent in non-MD group. The miR prediction analysis based on *LDLR* 3'UTR variants exclusive and/or most frequent in non-MD group showed that rs3180023, rs142697277, rs72658874 and rs7254521 variants decrease and/or break the binding of miR-193b-5p, miR-3152-3p, let-7a-2-3p, miR-211-3p, miR-4449, miR-501-3p, 502-3p and miR-5011-3p, while rs3180023, rs17243018, rs142697277, rs72658874 and rs7254521 variants also enhance and/or create the binding site for miR-5096, miR-1972, miR-4717-5p, miR-548a, miR-586, miR-5588-3p and miR-153. Regarding *APOB*, rs12720763 and rs72654428 decrease binding of miR-5000-5p, miR-769-5p and miR-4699-5p and break the binding site of miR-615-3p. For *PCSK9* rs149837083 variant decrease binding of miR-591 and miR-2278 with the target mRNA, while rs17111557 enhances binding and creates new binding sites of three miR (miR-4468, miR-4796-3p and miR-548t-5p). The 3'UTR variants in *LDLR, APOB* and *PCSK9* that reduce and/or break the binding of miRs may cause an increase in mRNA stability and those that enhance and/or create new binding sites of miRs may increase the mRNA degradation. **Conclusion:** The *in silico* predictive results suggest that variants in *LDLR*, *APOB,* and *PCSK9* 3'UTR have potential effects on interaction with regulatory miRs, and may explain epigenetic effects associated with FH, representing an important strategy to predict potential biomarkers and new therapeutic targets for FH.

B-004

Revisiting the Friedewald Equation: A Comparison of Directly Measured and Calculated LDL

C. S. Lau, S. P. Hoo, H. P. Gan, W. L. Quek, S. F. Yew, S. K. Phua, T. C. Aw. *CHANGI GENERAL HOSPITAL, Singapore, Singapore*

Background: LDL is calculated using the Friedewald equation - LDL-F(mmol/L) = [Total cholesterol(TC) – Triglycerides(TG)/2.2 – HDL]. However, LDL-F is invalid when TG≥4.52mmol/L (400.4mg/dL) and underestimates directly measured LDL (d-LDL) when LDL<1.8mmol/L. New AHA guidelines for secondary prevention of cardiovascular disease recommend lower LDL targets<1.8mmol/L (<70mg/dL). Thus, accuracy at low LDL levels is paramount. Although available, d-LDL has not entered clinical practice because of cost. However, the cost of d-LDL is now equal to HDL. We describe our experience with d-LDL versus LDL-F since 2017.

Methods: 84,365 samples were tested for d-LDL, TC, TG, and HDL on the CobasC702 (Roche); LDL-F was derived. Samples with TG≥4.52mmol/L were excluded (n=1489; 1.76%). d-LDL is a homogeneous colorimetric assay based on the breakdown of cholesterol by surfactants and cholesterol esterase. The analytical measuring range for d-LDL is 0.10–28.4mmol/L (3.9–1098mg/dL); this assay is unaffected by TG<22.6mmol/L (<2000mg/dL). We used MedCalc SoftwareV18.11 (Ostend, Belgium) for statistical analyses.

Results: Inter-assay imprecision (CV) for d-LDL was 1.31% (2.75mmol/L) and 1.33% (4.76mmol/L). d-LDL ranged from 0.10–19.46mmol/L (3.9–752mg/dL). LDL-F underestimated d-LDL across all levels of LDL in 98.1% of patients (81,336/82,876). As d-LDL increased, the difference between d-LDL and LDL-F (LDL-diff) increased; overall mean LDL-diff was 0.42mmol/L. The percentage LDL-diff (%LDL-diff) became more significant at lower levels of d-LDL; overall %LDL-diff was 15.8% (see TABLE 1). There was a stepwise increase in %LDL-diff as d-LDL decreased (≥4.91–1.30mmol/L); at d-LDL<1.29mmol/L %LDL-diff was 14.4%, possibly related to assay imprecision and more cases of LDL-F exceeding d-LDL. There was also a stepwise increase in LDL-diff with increasing TG.

Conclusion: LDL-F underestimates d-LDL at all levels of LDL including those <1.8mmo/L. The %LDL-diff is much larger at d-LDL levels <1.8mmol/L. With its superior accuracy, lower cost, and wide dynamic range, d-LDL should be incorporated into clinical practice.

TABLE 1: Mean difference and percentage difference of d-LDL and LDL-F at increasing d-LDL levels.

d-LDL mmol/L (mg/dL)	N	LDL-diff - mmol/L (range)	(95% CI)	Percentage LDL-diff (range)	(95% CI)
<1.30 (<50)	3439	0.18 (-0.58 to 0.95)	0.17 - 0.19	14.4 (-91.5 to 120.3)	12.57 - 16.18
1.30 - 1.79 (50 - 69)	9930	0.32 (-0.06 to 0.70)	0.32 - 0.33	20.4 (-4.2 to 45.0)	20.15 - 20.65
1.81 - 2.56 (70 - 99)	24973	0.39 (0.05 to 0.73)	0.39 - 0.40	18.0 (2.0 to 33.9)	17.86 - 18.06
2.59 - 3.34 (100 - 129)	21138	0.45 (0.07 to 0.83)	0.44 - 0.45	15.2 (2.3 to 28.1)	15.13 - 15.31
3.36 - 4.12 (130 - 159)	13963	0.48 (0.06 to 0.91)	0.48 - 0.49	13.1 (1.6 to 24.6)	13.01 - 13.21
4.14 - 4.89 (160 - 189)	6446	0.53 (0.10 to 0.96)	0.52 - 0.53	11.9 (2.2 to 21.5)	11.74 - 11.98
≥4.91 (≥190)	2987	0.57 (0.00 to 1.14)	0.56 - 0.58	10.3 (0.2 to 20.4)	10.12 - 10.49
Overall	**82876**	**0.42**		**15.8**	

**LDL Conversion factors: mmol/L x 38.66 = mg/dL; mg/dL x 0.0259 = mmol/L*

B-005

Development of a Direct Assay for Small Dense LDL (sdLDL) Assay on Roche Clinical Chemistry Analyzers

Y. Kaneoya[1], M. Ikaida[1], N. Satoh[1], Y. Hirao[1], J. Pelloli[2], H. Klima[3]. [1]*Denka Seiken Co. Ltd., Tokyo, Japan,* [2]*Roche Diagnostics International Ltd, Rotkreuz, Switzerland,* [3]*Roche Diagnostics GmbH, Penzberg, Germany*

Medical Background: sdLDL is an atherogenic lipoprotein. It has a lower affinity to LDL-C receptor leading to a higher retention time into the arterial wall and higher success ability of oxidation compared to large buoyant LDL particles. Clinical studies strongly suggest that elevated sdLDL concentration is associated with a risk of CHD irrespective of the presence of Diabetes leading to an increased CHD risk.**Test Principle:** Non-sdLDL lipoproteins, chylomicrons, VLDL, IDL, Large LDL and HDL are decomposed by a surfactant and sphingomyelinase in the pre-incubation phase of sample and Reagent 1. The cholesterol that is released from the non-sdLDL lipoproteins is then degraded. Cholesterol only from sdLDL particles is released by another surfactant in Reagent 2. Cholesterol esters and cholesterol from sdLDL are determined enzymatically by cholesterol esterase and cholesterol oxidase. The hydrogen peroxide released in the enzymatic reaction is detected by the 'Trinder Reaction' principle. **Development Goals sdLDL assay** Specimen: serum and plasma (K2-EDTA, Li-Heparin) high measuring range ≥ 2.587 mmol/L (100 mg/dL) Low interference by endogenous substances Reference interval study in accordance with Clinical Laboratory Standard Institute (CLSI) protocol EP28-A3cIn use time ≥ 28 daysShelf life ≥ 15 monthsFDA clearance for in vitro Diagnostic assay **Results:** ***Measuring range and lower limits of measurement*** The linear assay range of the sdLDL assay is from 0.1032.587 mmol/L (4.0100 mg/dL), LoQ ≤ 4.0 mg/dL. ***Traceability*** sdLDL assay has been standardized against the ultracentrifugation method. ***Limitations and interferences (CLSI Protocol EP07-A2)*** No interference of bilirubin (conjugated and non-conjugated) up to 1026 µmol/L (60 mg/dL), I-Index of 60, no interference of lipaemia (Intralipid) up to a L-Index of 1000, no significant interference from native triglycerides up to 13.7 mmol/L (1500 mg/dL), no interference of hemoglobin up to 621 µmol/L (1000 mg/dL), H-Index. No interference was found for drugs tested: Pravastatin, Pitavastatin, Atorvastatin, Rosuvastatin, Simvastatin, Fluvastatin, Ezetimibe, Fenofibrate, Gamma-Oryzanol, Bezafibrate, Probucol, Tocophenol Nicotinate, and Riboflavin Tetrabutyrate. ***Precision - CLSI EP05-A3 - 20 days*** Two controls, three human sera (low, middle, high): Total precision in all samples < 4,5 (%CV). ***Method comparison study*** There is a good correlation between sdLDL assay on cobas c 501 (x) and on Olympus AU 5800 systems (y). Passing/Bablok regression: y = 0.983x + 0.876 mmol/L, n = 110 serum samples.

Reference intervals Adults: (21-75 yrs.): 0.326 to 1.337 mmol/L (12.6 to 51.7 mg/dL). ***Regulatory*** sdLDL assay was FDA cleared (Reg. number: k161679). **Conclusions:** All development goals for the direct sdLDL assay were met. The use of the direct sdLDL method in laboratory routine will improve CHD risk assessment in patients as mentioned in the AACE guidelines 2017.

B-006

Lipoprotein Ratios better Predict Carotid Intimal Media Thickness (CIMT) than Individual Lipid Parameters in Nigerian Adult HIV Infected Individuals

L. C. IMOH[1], C. C. Ani[1], K. O. Iyua[1], A. I. Odo[1], M. S. Luka[1], C. S. Datiyi[1], D. O. Aina[1], K. O. Inaku[2], A. I. Affi[1], C. O. Isichei[1], B. N. Okeahialam[1]. [1]*JOS UNIVERSITY TEACHING HOSPITAL, JOS, Nigeria,* [2]*UNIVERSITY OF CALABAR, CALABAR, Nigeria*

Background: The increased risk of atherosclerotic cardiovascular disease is a major concern in HIV-infected individuals. Dyslipidemia is thought to play key role in the pathogenesis of atherosclerosis. Abnormal lipid fractions are common in HIV, but their role in atherosclerosis in this condition is still unclear. In view of previous conflicting reports regarding the association of lipids and atherosclerosis measured by Carotid-Intimal-Media-Thickness (CIMT) among Africans, we examined lipid-related ratios and individual lipid markers as predictors of CIMT in HIV-infected individuals.

Method: The survey population included randomly selected 148 stable HIV-infected adults aged 20 to 68 years who attended HIV clinics at Jos University Teaching Hospital and Faith Alive Foundation in Jos, Nigeria. All participants were assessed after overnight fasting for at least 12-hours. Lipid parameters included Total-Cholesterol (TC), Low-Density-Cholesterol (LDLc), High-Density-Cholesterol (HDLc), Triglycerides (TG) and lipid-related ratios. CIMT was measured as mean of bulb and Common Carotid segments of the Carotid Artery. Data were analyzed with the Statistical Package for Social Sciences (SPSS® Incorporated Chicago Version 18.0) software. P <0.05 was regarded as statistically significant.

Results: The study participants consisted of 105 (70.9%) female and the median (IQR) age was 41 (38-47) years. The median (IQR) CIMT was 0.65 (0.58-0.75) mm. CIMT was poorly correlated with individual lipid markers TC, HDLc, LDLc and TG (p>0.05). However, CIMT showed relatively higher but significant correlation with lipid ratios such as TC/HDLc, LDLc/HDLc and TC-HDLc/HDLc (P<0.05). Linear regression analysis showed that lipid parameters TC, HDLc, LDLc and TG did not significantly predict CIMT. However, TC/HDLc, LDLc/HDLc, TC-HDLc/HDLc significantly predicted CIMT as shown in table 1.

Conclusion: Lipoprotein ratios better predict CIMT compared to individual Lipid parameters in Nigerian Adult HIV-infected individuals. The use of Lipoprotein ratios should be incorporated into assessment of cardiovascular risk in HIV.

Table 1: Correlation and Regression analysis of Lipids and Lipoprotein ratios with CIMT

Parameters	r	P-Value	B (95%CI)	P-Value
TC	0.020	0.812	0.002 (0.015-0.019)	0.812
HDLc	-0.032	0.701	0.012 (-0.076-0.051)	0.701
LDLc	0.109	0.148	0.016 (-0.008-0.040)	0.187
TG	0.137	0.097	0.022 (-0.004-0.048)	0.097
TC-HDLc	-0.065	0.435	-0.006 (-0.019-0.008)	0.435
TC/HDLc	0.199	**0.015**	0.011 (0.002-0.008)	**0.015**
LDLc/HDLc	0.163	**0.047**	0.018 (0.001-0.035)	**0.045**
(TC-HDLc)/LDLc	0.214	**0.009**	0.011 (0.003-0.019)	**0.009**
Log(TG/HDL)	0.142	0.084	0.063 (-0.008-0.134)	0.084

B-007

Lipids by Nuclear Magnetic Resonance (NMR) Spectroscopy

H. F. Mohammad[1], C. Crumpler[1], F. C. Warf[2], E. Garcia[2], M. A. Connelly[2], E. Jeyarajah[2], D. W. Bennett[2], S. P. Matyus[2], D. N. Oskardmay[2], J. D. Otvos[2], R. M. Young[1]. [1]*LabCorp, Burlington, NC,* [2]*LabCorp, Morrisville, NC*

Background: Lipid testing is essential for cardiovascular disease (CVD) risk assessment and management of patients with established CVD. Most lipid assays are performed using conventional enzymatic/colorimetric methods on routine chemistry analyzers. For >20 years, nuclear magnetic resonance (NMR) spectroscopy has been used for the quantification of lipoproteins, and recently, a clinical NMR instrument, the Vantera® Clinical Analyzer, was developed and cleared by the FDA for high throughput clinical use. The Vantera is a robust clinical analyzer that uses small sample volumes, no reagents, and little sample preparation. The *NMR LipoProfile®* Test on the Vantera provides detailed measurements of lipoprotein particle concentrations and average particle sizes for VLDL, LDL, and HDL. The goal of this study was to implement the FDA- cleared Extended Lipid Panel (ELP) assay that simultaneously quantifies Total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL-C) and Apolipoprotein B (ApoB) to be used along with the *NMR LipoProfile* Test in the clinical NMR laboratory

Methods: The Extended Lipid Panel Assay was developed using Partial Least Squares Regression (PLS) and a large data set with both NMR and chemistry derived lipid data. The limit of quantification **(LoQ)** was determined as the lowest concentration measurable with acceptable precision and accuracy. **Within-run and within-laboratory precision** were determined according to EP-5A. Endogenous substances (n=8), normally found in blood, and exogenous substances (common and prescription drugs) (n=30) were evaluated for potential **interference** with Extended Lipid Panel Assay test results in accordance to CLSI EP7-A2 guidelines. A **method comparison** study was performed using serum samples across the reportable range

Results: Method comparison data, using linear regression, demonstrated excellent agreement with correlation coefficients (R) between NMR and chemistry values for TC, TG, HDL-C and ApoB of 0.994, 0.997, 0.985 and 0.980, respectively. Limits of quantification (LoQ) were determined to be 24 mg/dL for TC, 15 mg/dL for TG, 13 mg/dL for HDL-C and 18 mg/dL for ApoB. Within-run precision (%CV) ranged from 0.9-1.6, 1.0-1.0, 1.3-2.4 and 1.1-1.2, while within-laboratory precision ranged from 1.1-1.6, 1.0-1.4, 1.4-2.8 and 1.9-2.4, for TC, TG, HDL-C and ApoB, respectively. No major interferences were noted for the Extended Lipid Panel Assay. The measuring ranges for TC, TG, HDL-C and ApoB were 71-859 mg/dL, 36 to 939.0 mg/dL, 15 to 151.0 mg/dL and 36 to 344.0 mg/dL, respectively.

Conclusion: NMR is a robust, high-throughput platform for the quantification of both lipoprotein particle concentrations (LDL-P and HDL-P) as well as lipids in the clinical laboratory. Lipid measurements (e.g., TC, TG, HDL-C and/or ApoB) are often run using conventional enzymatic/colorimetric assays in a "routine" chemistry department that is physically separated from the NMR lab. Consolidating NMR and lipid testing onto one platform provided us with a noticeable improvement in testing turnaround time (TAT), significantly reduced reagent costs, and elimination of the potential for lab accidents and lost samples due to sample transfer between departments.

B-008

Clinical Significance of Apolipoprotein E-Containing HDL Cholesterol/Total HDL Cholesterol Ratio in Patients with Familial Hypercholesterolemia Diabetes

Y. Ito[1], M. Ogura[2], M. H. Shiba[2]. [1]*Denka Seiken Co.,Ltd., Tokyo, Japan,* [2]*National Cerebral and Cardiovascular Center Research Institute, Osaka, Japan*

Background: High-density lipoprotein (HDL) is classified into apolipoprotein E (apoE)-containing HDL (apoE-HDL) or apoE-deficient HDL. ApoE containing HDL accounts up to 10% of HDL in human adult plasma, reportedly exerting anti-atherosclerotic actions such as by enhanced cholesterol efflux from macrophages. We previously reported that decreased cholesterol efflux capacity (CEC) was an independent residual risk marker for the presence of atherosclerotic cardiovascular disease (ASCVD) in statin-treated heterozygous FH patients. However, the method for measuring CEC will never be standardized as a clinical laboratory test because of using cell lines and pooled serum/plasma of each institute. Therefore, further studies are needed to identify specific biomarkers which are common to both CEC and ASCVD risk, and we hypothesized plasma apoE-HDL cholesterol (apoE-HDL-C) may be a candidate. We aimed to investigate associations between apoE-HDL-C and CEC as well as clinical features including the presence of ASCVD in statin-treated FH.

Methods: 94 patients who are genetically diagnosed as FH were enrolled in this cross-sectional study. Thirty-four patients (36%) of them were known to have ASCVD. ApoE-HDL-C was measured using a homogeneous assay which we developed and reported previously (CCA 2016), and CEC was measured using radiolabeled cholesterol assay. As apoE-HDL-C is associated with total HDL-C, we adopted apoE-HDL-C/HDL-C ratio as a variable.

Results: There was no correlation between genotype of FH and apoE-HDL-C/HDL-C ratio despite apoE has a high binding affinity to the LDL receptor. ApoE-HDL-C/HDL-C ratio was negatively associated with Achilles tendon thickness as well as carotid intima-media thickness, but not related to the presence of corneal arcus. We found apoE-HDL-C/HDL-C ratio was positively associated with CEC (R=0.57, P<0.0001). Finally, apoE-HDL-C/HDL-C ratio was negatively associated with the presence of ASCVD independent of traditional cardiovascular risk factors even after adjustment for CEC (odds ratio per 1-SD increase, 0.26; 95%CI, 0.07-0.83; P = 0.0226).

Conclusion: ApoE-HDL-C/HDL-C ratio may be a good marker for residual risk of ASCVD in relation to CEC among statin-treated FH.

B-009

Application of Automated Homogenous Assay of LDL-triglycerides for Diagnosis of Non-Alcoholic Steatohepatitis

T. Sakurai[1], Y. Takahashi[2], K. Nouso[3], Y. Fujii[3], M. Oota[4], Y. Ito[4], A. Nagasaka[2], N. Wada[2], H. Chiba[1], S. Hui[1]. [1]*Hokkaido University, Sapporo, Japan,* [2]*Sapporo City General Hospital, Sapporo, Japan,* [3]*Okayama University, Okayama, Japan,* [4]*Denka Seiken Company Limited, Tokyo, Japan*

Background: Low-density lipoprotein-triglycerides (LDL-TG), which is total triglycerides carried in LDL, have been reported to reflect an atherogenic potential of LDL than LDL-cholesterol (LDL-C) (März W et al., Circulation, 2004). Previously we have reported that the content of TG-rich LDL increases in severe liver disease (Sakurai T et al., Ann Clin Biochem, 2012). Although non-alcoholic steatohepatitis (NASH) may progress to end-stage liver disease, it remains unknown if LDL-TG level increases in NASH. Despite the increase in its global burden, no efficient blood biomarker for the diagnosis and prediction of NASH has been established. In current practice, there are no reliable investigations other than invasive technique like histological examination of the liver biopsy. Thus, the development of specific blood biomarker for NASH is eagerly needed to improve the clinical outcome and reduce the economic burden. Here, a ratio of TG/cholesterol was determined in the LDL fraction separated using gel-filtration HPLC from the serum of NASH patients. We also investigated the performance of an automated homogenous assay for LDL-TG, developed recently by Denka Seiken Co. Ltd., to distinguish between NASH and simple steatosis.

Methods: The LDL particles were isolated from the fresh fasting serum of healthy subject (n = 12), hypertriglyceridemia (n = 9), and NASH (n = 10) using HPLC system equipped with the Superose 6 column (Sakurai T *et al.*, Ann Clin Biochem, 2012). Eluates were used to analyze TG and total cholesterol by enzymatic assay and the area under the curve was plotted based on the amounts of TG or cholesterol in each LDL fractions. Next, we compared TG and cholesterol content in LDL from NASH (n =

35) and simple steatosis (n = 9) using the automated enzymatic assay of LDL-TG and LDL-cholesterol.

Results: The TG/cholesterol ratio of LDL fraction in NASH was significantly higher than that in other groups, suggesting that LDL in NASH are rich in TG. The area under the ROC curve was 0.800 ($P < 0.05$), indicating that TG/cholesterol ratio of LDL can distinguish between the patients with hypertriglyceridemia and NASH. In the Both LDL-TG and TG/cholesterol ratio in LDL were significantly increased in NASH compared to simple steatosis ($P < 0.05$; $P < 0.01$, respectively). The area under the ROC curve of the TG/cholesterol ratio was 0.857 ($P < 0.01$), indicating that the ratio can also distinguish between NASH and simple steatosis.

Conclusion: We applied an automated homogenous assay to determine LDL-TG in NASH. The NASH patients had significantly increased serum LDL-TG and LDL-TG/LDL-C ratio compared to simple steatosis. LDL-TG can be a promising diagnostic biomarker to distinguish NASH and simple steatosis.

B-010

Mean Platelet Volume in Patients with Increased Lipoprotein a Level

S. Cho, Y. Kim, H. Gu. *Kyung Hee University Hospital, Seoul, Korea, Republic of*

Background: Because the prothrombic role of lipoprotein (a) or Lp (a) can also result from promotion of platelet activation besides inhibition of fibrinolysis, we made a hypothesis that platelet index can be changed in conditions related with increased Lp (a). Therefore, we planned to analyze mean platelet volume (MPV) in patients with increased levels of Lp (a) to investigate whether thrombogenicity of Lp (a) can affect platelet activation index such as MPV.

Methods: We reviewed the total 1977 test results of Lp (a) done in our hospital from January 2013 and April 2014. This study finally enrolled 290 patients with increased Lp (a) results above 1.5 times higher than the upper reference limit (30 mg/dL). As a control group, 143 individuals were randomly selected among who visited in our hospital for medical check-up and they were used in our previous study.

Results: The mean age of patient group was 65.78 (range 12-91) years and male to female ratio was 132: 158. In patient group, mean of Lp (a) and MPV were 74.03 (range 45-175) mg/dL and 7.31 (range 5.8-10.0) fL, respectively. We found that MPV was significantly decreased in patient group comparing with control group (7.96 fL, P < 0.0001, Fig. 1).

Conclusion: This finding can mean that reactive platelets with large size be preferentially consumed in active endothelial lesion such as atherosclerotic plaque. It seems that thrombogenicity of Lp (a) can contribute to this phenomenon. In the future, clinical meaning of decreased MPV should be further investigated whether it is directly related with start or growth of atherosclerotic lesion in patients with high Lp (a) level. Moreover, it is recommended to evaluate whether therapeutic lowering of Lp (a) by statin or niacin treatment, or apheresis, can lead to normalization of MPV and stabilization of atherosclerotic lesion.

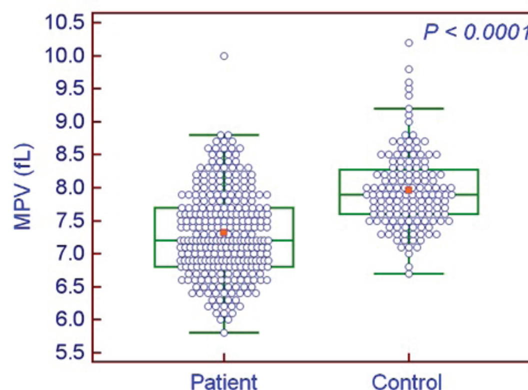


Figure 1. Mean platelet volume (MPV) in patients with increased Lp (a) level. Comparing with control group (7.96 fL, N=143), patient group (7.31 fL, N=290) showed a significantly decreased MPV level ($P < 0.0001$). MPV was measured in Advia 2120 (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Lp (a) tests were performed with Siemens N Latex Lp (a) Reagent (Siemens Healthcare Diagnostics, Marburg, Germany).

B-011

Increased Angiopoietin Like Protein 4 (ANGPTL4) is Associated with Higher Concentration of LDL-Triglycerides in Type 2 Diabetes

Y. Ito[1], H. Nagaike[2], T. Hayashi[2], T. Hirano[2]. [1]*Denka Seiken Co.,Ltd., Tokyo, Japan,* [2]*Showa University School of Medicine, Tokyo, Japan*

Background: Diabetic dyslipidemia is typically characterized by an increase in plasma triglycerides (TG), small dense (sd) LDL-C and a concomitant increase in TG contents in LDL (LDL-TG). LDL-TG, a sub-fraction of TG has been reported elevated and to be a more powerful risk factor than LDL-cholesterol. Angiopoietin-like protein (*ANGPTL*) *3* and *ANGPTL4* are associated with increased plasma TG levels due to their roles in regulating the activity of lipoprotein lipase in circulation. However, the association between *ANGPTL3, 4* and LDL-TG are unclear. We examined the association between the plasma level of *ANGPTL)3*, *ANGPTL4* and level of LDL- TG in patients with type 2 diabetes (T2DM).

Methods: 126 diabetic patients (male/female 102/24) were enrolled into the study. We measured LDL-TG and sd LDL-C by newly developed homogeneous assays (Denka Seiken) and concentrations of large, buoyant (lb) LDL-C were estimated by subtracting the sd LDL-C from the LDL-C. Serum *ANGPTL3* and *ANGPTL4* levels were measured by ELISA kits (Immuno-Biological Laboratories) in 126 T2DM patients (male/female 102/24). Preheparin lipoprotein lipase (LPL) mass were measured by sandwich ELISA. Waist circumference (WC), visceral fat (VF) and subcutaneous fat (SF) measurements at the umbilicus level were performed on CT, and WC, VF and SF areas were calculated using a workstation, Ziostation (Ziosoft Inc., Tokyo).

Results: In univariate analysis, LDL-TG was positively correlated with sd LDL-C and *ANGPTL4*, and negatively correlated with preheparin LPL mass. However LDL-TG was not correlated with *ANGPTL3.* Multiple linear regression analysis showed that *ANGPTL4* and preheparin LPL mass were independent predictors of LDL-TG concentrations.

Conclusion: Higher plasma levels of *ANGPTL4* but not *ANGPTL3* was closely associated with higher levels of LDL-TG. The findings suggest that *ANGPTL4* was related to LDL-TG-enrichment as a powerful cardiovascular disease risk marker in T2DM.

B-012

Performance Evaluation of Five Lipoprotein(a) Immunoassays on the Roche Cobas c501 Chemistry Analyzer

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

Objectives: Measurement of lipoprotein(a) [Lp(a)] has been used to aid in risk assessment of atherosclerotic cardiovascular disease (ASCVD). While standardization and harmonization of methods is important to the medical community, traceability information for Lp(a) assays is generally not listed in Lp(a) package inserts. The aim of currently study is therefore to evaluate performance characteristic of 5 different Lp(a) assays using the cobas c501 (Roche Diagnostics) analyzer with a particular focus on method comparison experiments

Methods: Lp(a) was evaluated using the Roche LPA2 assay and four open channel Lp(a) assays (Diazyme, Kamiya, MedTest, Randox). Studies included limit of detection (LOD), imprecision, linearity, method comparison, and evaluation of healthy subjects. Imprecision (intra-day, 20 replicates; inter-day, duplicates twice daily for 5 days) and linearity were evaluated using patient pools. Linearity required a minimum of 5 patient splits spanning the analytical measurement range of each assay. Method comparison used 80 residual serum samples with median concentrations ranging from (7.4 – 67.8 mg/dL). Reference intervals included 120 self-reported healthy subjects (61 females/59 males).

Results: LOD determination met manufacturers' claims. Imprecision studies demonstrated CVs ranging from 2.4 – 5.3% for the low pool (average concentration ranging from 7.16 – 12.43 mg/dL) while the high pool had CVs ranging from 0.8 – 3.0% (average concentrations ranging from (27.64 – 50.42 mg/dL). All assay were determined to be linear. Summary of regression analyses of samples compared to a) all method median and b) Diazyme is provided (Table 1). Evaluation of apparently healthy individuals resulted in a slightly higher number of individuals with Lp(a) concentrations >30 mg/dL for both Diazyme and MedTest assays. The upper reference limit for females was higher than males for all assays evaluated.

Conclusions: All assays performed well compared to specified manufacturers performance characteristics, although overall lack of agreement among Lp(a) assays still exist.

Table 1. Method Comparison

Comparison to all method median (n=63)					Comparison to all method median with results <30 mg/dL (n=39)				
Method	Slope	Intercept	R	% Bias	Method	Slope	Intercept	R	% Bias
Diazyme	1.288	0.404	0.9921	26.20	Diazyme	1.373	-1.02	0.9741	23.77
Kamiya	0.800	-0.179	0.9861	-23.02	Kamiya	0.782	0.33	0.9520	-20.26
MedTest	1.217	0.986	0.9905	22.24	MedTest	1.274	0.12	0.9878	24.99
Randox	0.963	-0.034	0.9975	-3.94	Randox	0.991	-0.06	0.9949	-1.50
Roche	0.968	-3.683	0.9905	-16.94	Roche	0.741	0.13	0.9910	-28.05
Comparison to Diazyme (n=63)					Comparison to Diazyme with results <30 mg/dL (n=39)				
Method	Slope	Intercept	R	% Bias	Method	Slope	Intercept	R	% Bias
Kamiya	0.620	-0.41	0.9864	-48.49	Kamiya	0.559	1.04	0.9283	-43.51
MedTest	0.945	0.62	0.9920	-4.02	MedTest	0.930	1.03	0.9711	1.24
Randox	0.746	-0.28	0.9858	-30.06	Randox	0.722	0.68	0.9725	-25.24
Roche	0.747	-3.81	0.9706	-42.67	Roche	0.534	0.75	0.9617	-50.97

B-013

Small Dense Low-Density Lipoprotein Cholesterol Predicts Carotid Intimal Medial Thickness Progression

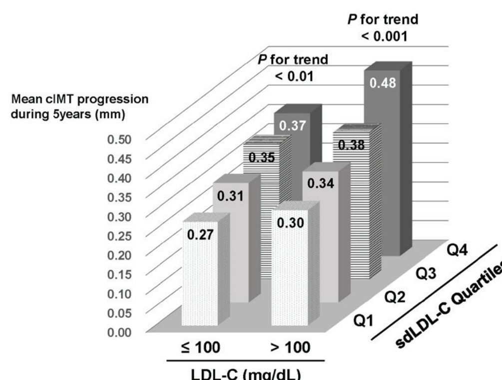
H. Ikezaki[1], N. Furusyo[1], Y. Ito[2], Y. Yokota[3], M. Ai[4], B. F. Asztalos[5], M. Murata[1], E. J. Schaefer[5], J. Hayashi[6]. *[1]Kyushu University, Fukuoka, Japan, [2]Denka Seiken Co. Ltd., Niigata, Japan, [3]Showa University, Tokyo, Japan, [4]Tokyo Medical and Dental University Hospital, Tokyo, Japan, [5]HNRCA AT TUFTS UNIVERISTY, Boston, MA, [6]Haradoi Hospital, Fukuoka, Japan*

Background: Low-density lipoprotein cholesterol (LDL-C) and small-dense LDL-C (sdLDL-C) are atherogenic lipoproteins associated with increased cardiovascular disease (CVD) risk and carotid intimal medial thickness (cIMT). Our objectives were to assess LDL-C, sdLDL-C and other lipoproteins with regard to cIMT progression in participants in the prospective Kyushu Okinawa Population Study (KOPS).

Methods: Plasma total cholesterol, LDL-C, sdLDL-C, LDL-triglycerides, high density lipoprotein cholesterol (HDL-C), HDL2-C, HDL3-C, triglycerides, lipoprotein(a), and adiponectin were measured in 2,030 men and women (median age 59 years) who had cIMT measured baseline and after 5 years of follow-up surveys. Subjects on cholesterol lowering medication were excluded. Univariate and multivariate regression analyses and least square methods were performed to examine the relationship between LDL-C, sdLDL-C, other lipoproteins and cIMT progression.

Results: Median cIMT at baseline was 0.63mm and median cIMT progression during 5 years follow-up was 0.18mm. After adjustment for standard CVD risk factor included age, sex, systolic blood pressure, total cholesterol, HDL-C, smoking, diabetes, hypertension, and hypertension treatment, only LDL-C, sdLDL-C, and body mass index were significantly associated with cIMT progression. Even in individuals with LDL-C < 100 mg/dl, considered to be optimal, elevated sdLDL-C were associated with cIMT progression (P = 0.009) in a model including all other established CVD risk factors.

Conclusion: Both sdLDL-C and LDL-C are significant predictors of cIMT progression, and measurement of sdLDL-C may add significant information to formulate optimal therapy for CVD prevention.



B-015

A Cohort Investigation on the Incidence of Metabolic Syndrome in Health Examination Population in One Hospital in China from 2011 to 2018

D. Yang, Y. Mei. *The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: Metabolic syndrome has become a worldwide concern in recent years. Current incidence investigations on metabolic syndrome were mostly cross-sectional study and there were few long terms cohort design. This study investigates the relationship between the incidence rate of metabolic syndrome and age, gender and level of uric acid in a cohort population.

Methods: Data from population who had annual health check up in one hospital in China from 2011 to 2018 were retrospectively analyzed. The diagnostic criteria from the Chinese Diabetes Society was used to diagnose metabolic syndrome and the incidence rate of metabolic syndrome was investigated for this population.

Results: A total of 1908 male and 1909 female people who receiving health examination were included. The average incidence rate of metabolic syndrome from 2011 to 2018 was 0.93% for those males who were younger than 25 years old and it increased to 12.94% for those males who were 45-49 years old and it kept around 11.71%-19.17% for those males over 50 years old. The average incidence rate of metabolic syndrome from 2011 to 2018 was low (0.09%-0.14%) for those females who were under 39 years old and it increased obviously for those females who were 40-69 years old (2.10%-20.11%). The cumulative time of metabolic syndrome increased along with the cohort time. Among them, 9.85% males and 3.82% females had metabolic syndrome for no less than four times. The main types of metabolic syndrome were obesity, hyperlipidemia, and hypertension and it accounted for 42.36% in male and 39.75% in female. The incidence rate of metabolic syndrome was related to the level of uric acid. The average incidence rates for those males with low to high quartile of

average uric acid from 2011 to 2018 were 7.63%, 8..48%, 10.67% and 14.90%. Those numbers were 1.30%, 2.09%, 3.81% and 10.26% for those females respectively.

Conclusion: The incidence rate of metabolic syndrome are closely related to age, gender, cohort time and uric acid level. Appropriate preventive and treatment measures should be adopted based on the incidence rate of metabolic syndrome.

B-017

Clinical and Laboratory Characteristics of Children with Genetic Diagnosis of Familial Hyphercholesterolemia from Hipercol-Ceara, Brazil Program

M. C. G. CASTELO[1], M. T. A. Sales[2], L. F. F. Aragão[2], G. C. Nogueira[2], L. A. A. Batista[2], C. E. Jannes[3], A. B. de Carvalho[2], R. M. Montenegro Júnior[2], R. D. Santos Filho[3], A. C. Pereira[3], G. A. Campana[4]. *[1]DASA, FORTALEZA, Brazil, [2]UFC, FORTALEZA, Brazil, [3]USP, São Paulo, Brazil, [4]DASA, São Paulo, Brazil*

Background: Familial hypercholesterolemia (FH) is a cause of monogenic primary dyslipidemia, causing elevated low-density lipoprotein (LDL-C) levels even in early ages. Cascade screening programs can identify family mutations and undiagnosed cases, also can contribute with data about this disease, especially in children. **Objective:** This study aimed to describe the clinical and laboratory characteristics of children diagnosed genetically with FH by HIPERCOL-CE program. **Methods:** This cross-sectional study evaluated children from HIPERCOL-CE program from 2013 to 2019. A total of 37 children referred for clinical suspicion of FH were genetically investigated. The mutation in LDLR receptor gene (LDLR) was present in 19 children (51.3%) witch 6 of them were index cases. The children were evaluated for the presence of family history of premature cardiovascular disease (PCVD), clinical signs suggestive of FH and anthropometric analyis. **Results:** We evaluated 19 children (8 female and 11 male gender), median age was 6.8 years. The clinical and laboratory characteristics are described in table 1. **Conclusions**: In this pediatric population most of the children were eutrophic and without suggestive clinical findings to FH diagnosis. Positive family history and LDL-C levels were the main findings for suspected FH diagnosis. Because of the scarce presence of clinical findings in the pediatric group, it is important to emphasize that the cascade screening to allows the timely diagnosis of children at high risk of PCVD.

Clinical and laboratory characteristics of children with genetic diagnosis of Familial Hyphercholesterolemia from Hipercol-Ceara, Brazil Program	
Sex (male/female)	11/8
Median age at first consultation of the program (years)	6.8
Family history of PCVD	5
Cardiovascular event (isquemic cerebrovascular event)	1
Prevalence of clinical findings (%) Corneal arch Xanthoma Xanthelasma	0 5 5
Median height (SDS)	-0.49
BMI (SDS)	0.90
WC > 90th percentile < 90th percentile	3 10
Median LDL-c (mg/dL and mmol/L) at baseline evaluation	209/5.40
PCVD: premature cardiovascular disease; BMI: Body Mass Index; SDS: Standart deviation; WC: waist circunference; LDL: Low density lipoprotein	

B-018

Genetic Profile of Children with Familial Hypercholesterolemia from the Hipercol Program, Ceara, Brazil

M. C. G. CASTELO[1], R. D. Santos Filho[2], C. E. Jannes[2], A. C. Pereira[2], G. C. Nogueira[3], L. A. A. Batista[3], A. D. R. Montenegro[3], R. M. Montenegro Júnior[3], G. A. Campana[4]. *[1]DASA, FORTALEZA, Brazil, [2]USP, São Paulo, Brazil, [3]UFC, FORTALEZA, Brazil, [4]DASA, São Paulo, Brazil*

Background: Familial hypercholesterolemia (FH) is an autosomal dominant disease, caused by mutations in the low density lipoprotein receptor (LDLR) gene, apolipoprotein B (APOB) gene or proprotein convertase subtilisin/kexin type 9 gene (PCSK9). The diagnosis of FH in children and adolescents is much more challenging, because early in life, the disease is asymptomatic. **Objective:** This study aimed to describe the genetic data obtained of children from HIPERCOL, Ceara, Brazil program. **Methods:** This cross-sectional study evaluated children with genetic diagnosis of FH from the cascade screening program from 2013 to 2019. The mutation detection was initially made by LDLR gene sequencing. If a disease causing mutation was not found, the PCSK9 (Exon 7) and APOB (part of exon 26) genes both considered as hotspots were studied. In the case of a negative result in all three genes, the MLPA technique was used to search for deletions or insertions in the LDLR gene. Sanger sequencing was performed in the ABI3500xl sequencer and the sequences were analyzed with the software SeqMan. **Results:** We evaluated 19 children (8 female and 11 male), mean age was 9 years old (±5,25). All of them presented LDLR gene mutation (Table 1). A total of 13 children inherited the mutation from one parent and 6 children inherited the mutation from both parent (compound heterozygous). One of those children presented numerous xanthomas. The LDL levels were 63.08mmol/L for simple heterozygous cases and 68.33 mmol/L for the compound heterozygous cases. **Conclusion:** Only mutations in receptor (LDLR) gene were present in all children. The simple heterozygous form was the most prevalent. The only clinical characteristic was high LDL-C levels. Physical signs were uncommon and most of children were asymptomatic.

Location LDLR gene	**Aminoacid change**	**N=19**
Exon 4	p.Arg115Cys	3[a]
Exon 5 e Exon 6	Duplicação	1
Exon 7	p.Ser326Cys	4[a]
Exon 7	p.Ser326Cys	1
Exon 8	p.Gly373Asp	4[a]
Exon 8	C392Y(Cys392Tyr)	3
Exon 9	p.Arg406Trp	1
Exon 9	A431Ala431Thr	3
Exon 10	p.Val523Met	1
Exon 16	p.lle792Metfs*137	2
Exon 16	L792MfsX136 (lle792MetfsX136)	2[a]

Table 1 Mutations found in 19 children by LDLR gene sequencing.

[1] Brothers compound heterozygote with the same gene mutation.

[2] A child have two gene mutations.

B-019

Checkmate: Evaluating the Cardio Check POC Device for Accurate Determination of Lipid Profiles and Cardiovascular Risk in Ambulatory Patients

E. Schuler, K. Blakemore, D. Paskovics, A. Woodworth. *University of Kentucky, Lexington, KY*

Background: The ACC/AHA guidelines recommend lipid profile screening to assess risk for developing cardiovascular disease (CVD) and identify patients needing therapeutic intervention. The use of Point of Care (POC) devices for the evaluation of lipid profiles offers an attractive alternative to central laboratory testing, particularly for health and wellness screening in the ambulatory setting. POC devices, however, may not provide the robust analytical performance characteristics required for lipid assays involved in risk stratification for cardiovascular disease. In this study we compare the analytical performance and utility of the POC Cardio Check (CC) and central laboratory Roche Cobas lipid assays in assessing CVD risk among ambulatory patients.

Objective: To evaluate the utility of the Cardio Check POC device to measure lipids and assess cardiovascular risk in an ambulatory patient population.

Methods: A sample of volunteers representing an ambulatory population was recruited. Blood samples were collected by both finger stick (capillary) and venipuncture. Lipid profiles including total cholesterol (TC), HDL, triglycerides (TRIG) and calculated LDL (LDL-C) were determined for each sample by the CC and Roche Cobas assays. Statistical analysis assessing bias and stability of samples for the CC system was performed in EP Evaluator. The resulting bias was extrapolated to a retrospective cohort of all ambulatory patients with lipid profiles performed at the University of Kentucky Medical Center (UK) clinical laboratories for a 6 month time period. Population data was categorized according to risk thresholds defined by current 2018 ACC/AHA guidelines.

Results: Significant biases were observed in lipid results generated from capillary (C) and venous (V) whole blood specimens on the CC compared to venous plasma specimens measured on the Roche Cobas. Compared to the Roche results, the cardio check biases were (C) -20% and (V) -28% for TC; (C) <3% and (V) 5% for HDL; (C) 12% and (V) 12% for TRIG; and (C) -35% and (V) -45% for LDL-C. Among the ambulatory cohort of 13,393 patients with 13,986 lipid profiles, 28% and 55% had optimal TC (≤150 mg/dL) and LDL-C (≤100 mg/dL), respectively. Cardio Check biases were applied to this ambulatory population and the percent of outpatients with optimal TC increased from 28% to 64% (Δ= +36%) and 79% (Δ= +41%) when applying CC capillary and venous bias, respectively. The effect of CC biases on calculated LDL was similar, with significant increases in the percentage of patients with optimal LDL from 55% to 94% (Δ= +39%) and 98% (Δ= +43%) when applying CC capillary and venous bias.

Conclusions: The Cardio Check lipid assays demonstrated variable performance when compared to central laboratory methods. Cardio Check HDL results were more accurate than TC and TRIG, perpetuating error in calculations for LDL. Importantly, the Cardio Check system demonstrated a significant negative bias for both total and calculated LDL cholesterol, significantly under estimating CVD risk and candidates that should be considered for cholesterol-lowering therapeutics. Ambulatory providers should exercise caution when interpreting lipid profiles evaluated by POC devices to determine CVD risk, particularly in the absence of baseline measurements evaluated by central laboratory methods.

B-020

Analytic Interference of Hypertriglyceridemia in Infant with Clinical Diagnosis of HyperKylomicronemia

M. C. G. CASTELO[1], F. M. S. Coelho[2], L. B. Herculano[2], E. M. Ribeiro[2], M. T. A. Sales[3], L. L. A. Cavalcante[1], G. A. Campana[4]. *[1]DASA, FORTALEZA, Brazil, [2]Hospital Albert Sabin, FORTALEZA, Brazil, [3]UFC, FORTALEZA, Brazil, [4]DASA, São Paulo, Brazil*

Background: Hypercholesterolemia and familial hyperkylomicronemia are the only abnormalities that are completely expressed in childhood, and others are more rarely recognized. The latter is characterized by very high levels of triglycerides, which in turn through marked lipemia can act as an analytical interferer. Objective: Describe the clinical case in which elevated levels of triglycerides interfered in the study of other analytes in a infant whit familial hyperkylomicronemia diagnosis. Description: Infant of one month and fifteen days of life, without pathological antecedents, investigating infectious disease (vomits and fever), presented blood sample of pink coloration (Figure 1), which was not possible to be analyzed due to the intense lipemia. In a new sample, after serum dilution, triglyceride (TG) levels were 4,440mg / dL and total cholesterol (TC) of 1257mg / dL, and it was then possible to perform the other hemogram tests. The infant was diagnosed with familial hyperchylomicronemia. In physical examination, the only clinical finding was the presence of a small xanthoma in the face (Figure 1). After dietary changes, TG levels are 650mg / dL and CT 389mg / dL. Conclusion: Spectrophotometric analysis can be influenced by lipoprotein particles that, by absorbing light, impair the uptake by other analytes and thus those quantification. The report emphasizes the importance of medical knowledge regarding pre-analytical, analytical and post-analytical interferences in clinical practice.

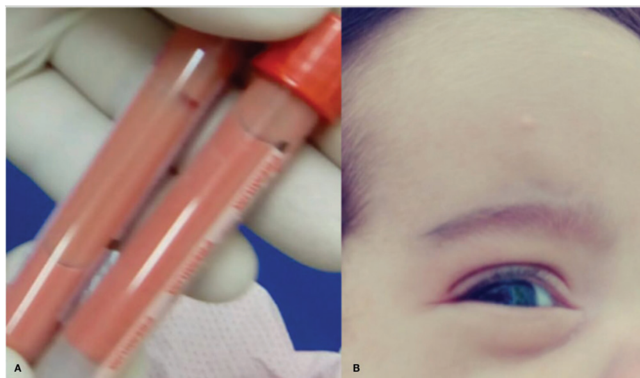


Figure 1 (A) Pink serum. (B) Xanthoma.

B-021

Fine-Tuning Lipid Panel Reflex Testing Algorithm for Direct LDL Cholesterol Measurement

S. Lo, H. Yu. *Geisinger, Danville, PA*

Background: The Friedewald equation has been shown to underestimate calculated LDL cholesterol (LDL-C) at low concentrations in the presence of elevated triglyceride. With recent AHA/ACC guidelines focusing on using LDL-C as a treatment target, with 70 mg/dL for patients with very high ASCVD risk, it is important for laboratories to improve the accuracy of LDL-C near this medical decision point. Here, we used a data-driven approach to optimize reflex parameters that allowed us to improve the accuracy of LDL cholesterol reported from our lipid panel.

Methods: Optimized reflex parameters for LDL-C were determined using 1189 lipid panel results generated during a 2-week period from in-house lipid panel with reflex to direct LDL-C measurement. Lipid panel (cholesterol, HDL cholesterol, and triglyceride (TRIG)) were measured using Roche Cobas c702. Direct LDL-C, also measured using Roche Cobas c702, was reflexively measured when TRIG exceeded 150 mg/dL. Bias between calculated and direct LDL-C was analyzed, using direct LDL-C as the reference method. Data was stratified based on TRIG (150-199, 200-249, 250-299 and 300-399 mg/dL) and calculated LDL-C (<70, 70-99, 100-129, 130-159, 160-190, >190 mg/dL).

Results: An average negative bias of 10 to 25% was observed for calculated LDL-C <70 mg/dL at all TRIG concentrations. Similarly, an average negative bias of 10 to 15% was observed for calculated LDL-C between 70-99 mg/dL when TRIG was above 200 mg/dL. For all other LDL-C and TRIG groups, the average negative bias was determined to be less than 10% for LDL-C. With these findings, we propose a reflex algorithm for direct LDL-C measurement when calculated LDL-C is <70 mg/dL and TRIG is > 150 mg/dL or when calculated LDL-C is 70-99 mg/dL and TRIG is >200 mg/dL.

Conclusion: Lipid panels that report Friedewald calculated LDL-C should consider optimizing their reflex testing algorithm to direct LDL-C measurement to improve LDL-C accuracy and mitigate undertreatment of high-risk patients.

B-022

Association between Expression of 25 Genes and DNA Methylation in CARDIA

M. D. Gross[1], Y. Zheng[2], L. Hou[2], D. Jacobs[1], W. Guan[1], B. Thyagarajan[1]. *[1]University of Minnesota, Minneapolis, MN, [2]Northwestern University, Chicago, IL*

Background: Gene expression regulation by the DNA methylome requires further research in population samples.

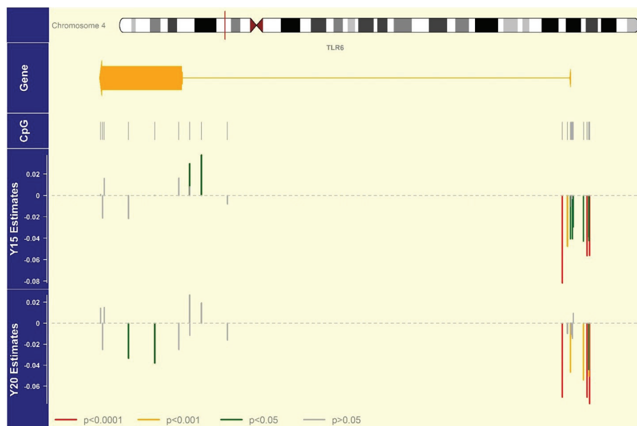
Methods: Using NanoString technology in RNA isolated from PAXGene tubes at CARDIA Y25 (2010-11), we assayed RNA expression of 25 genes in 3074 black and white men and women, average age 50. The methylome was assessed at over 850,000 CpG sites in CARDIA Y15 (n=912) and Y20 (n=835) of these people. We examined 589 CpG sites nearby the 25 genes (from 1500 bp upstream of transcription start site to

transcription end site) for cis-meQTL analysis. For each each CpG and each CpG-targetting gene expression combination we regressed gene expression on methylation status, adjusting for age, race, sex, center, methylation sample cell type proportion, and batch. Y15 and Y20 methylation data were analyzed separately.

Results: Across 10 genes, 33 CpG sites had associations with gene expression, satisfying Bonferroni corrected significance of 0.00008; these were consistent for both Y15 and Y20 analyses. Several genes were related to inflammation, namely TLR1 (2 significant out of 12 CpG sites), TLR5 (1/33), TLR6 (3/22), IL1RN (2/20), CCR1 (4/13), TNF (8/26), and TGFA (5/43), and several other genes, namely SPP1 (2/8), GSTT1 (4/4), and ALOX15 (2/29). Findings for TLR6 are shown (Figure) as an example. The majority of the significant CpGs were enriched in the gene promoter region and inversely associated with gene expression. Gene body CpGs had positive association with gene expression.

Conclusion: Many cis-methylation sites are consistently associated with gene expression measured 5 and 10 years later, consistent with long-term regulation of inflammatory genes.

Figure: Associations of extent of TLR6 expression with extent of methylation of CpG islands nearby the TLR6 gene (# of CpGs tested: 22)


Chromosome 4
TLR6
Gene
CpG
Y15 Estimates
0.02
0
-0.02
-0.04
-0.06
-0.08
Y20 Estimates
0.02
0
-0.02
-0.04
-0.06
p<0.0001
p<0.001
p<0.05
p>0.05

Wednesday, August 7, 2019

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

Management Sciences and Patient Safety

B-023

Analysis of the Changing Patterns of On-Call Consultations Among Clinical Pathology Trainees in a Referral Center

F. S. TEMPLO. *PHILIPPINE HEART CENTER, QUEZON CITY, Philippines*

Background: On-call consultations received by the clinical pathology trainees provide relevant insights into the most common problems encountered by the laboratory technologists and other healthcare personnel relying on the laboratory results. This study aims to identify the changing pattern of on-call consultations received by the clinical pathology trainees in terms of the total number of calls, subjects and type of callers between 2 periods spanning 12 years. **Methods:** Review of all documented on-call consultations received from 5pm to 8am on weekdays and all days on weekends among clinical pathology trainees for two 12 month-periods (April 2005- March 2006 and April 2017-March 2018) was performed. The consultations were categorized according to the call subjects and types of caller. **Results:** In 2005-2006, a total of 97 on-call consultations were received and acted upon by the residents. The three most common on-call consultations were request for blood smear review (29.6%), release of blood products/supply issues (18.5%) and panic values and interpretation of clinical chemistry results (11%). The calls came from laboratory technologists (91%), physicians (5%) and nurses (4%).In 2017-2018, the total number of on-call consultations increased to 148 (up by 52.6%).The three most common on-call consultations were request for blood smear review (32.8%), panic values and interpretations of clinical chemistry results (25.7%) and release of blood product/supply issues (17.6%). The calls still came mainly from the laboratory technologists (72%) with increasing proportions from physicians (15%) and nurses (12%). **Conclusion:** The changing patterns of on-call consultations among clinical pathology trainees over time signify the broadening and increasingly relevant role of the clinical laboratory in the acute care settings in the hospitals. Continuous monitoring and analysis of these changes could be a potential quality assurance tool, a valuable learning opportunity for the residents, and a platform for assessment of customer satisfaction.

B-024

Does the High Prevalence of Cardiovascular Risk Factors Confirms the Epidemiology Transition in Developing Countries? The Case of Mali

M. M. Coulibaly, M. Barry, B. Sonfo, L. Sidibé, B. Diabaté, M. Diawara, D. Samaké, S. Bah, B. Maiga. *University of Technical Sciences and Technology of Bamako, Mopti, Mali*

Background: Nowadays, developing countries are facing to the epidemiology transition whereas health system remains weak and unprepared to prevent and manage cardiovascular disease which accounts for a significant share of premature deaths in low- and middle-income countries. The overall objective of this study was to assess the risk factors of cardiovascular in heath workers.

Methods: We carried out a cross-sectional study from April to December 2017 to accessed cardiovascular risk factors among 292 health workers aged from 25 to 70 old. We measured physical and biological cardiovascular risk factors to determine their specific prevalence and above all the overall risk according to the Framingham and European SCORE.

Results: The prevalence of cardiovascular risk factors was 36.64%, 21.57%, 18.84%, 14.04%, 13.01% respectively for high blood pressure, hyperglycemia, obesity, smoking and alcohol consumption. Metabolic syndrome accounts for 23.63%. The overall risk was 26.33% and 8.57% according to the Framingham and SCORE algorithms respectively. Metabolic syndrome was significantly higher among women than men, 28.41% against 16.38% (kh2 = 4.96, p = 0.02). In contrast, the overall risk was higher among men at 49.54% compared to women 7.84% (hk2 = 56.53 and p < 0.001) and 14.67% against 4.00% (kh2 = 4.93 and p = 0, 03) according to Framingham and SCORE algorithms respectively. The same trend was observed between subjects aged 50 and those under 50 years old, 34.46% versus 13.16% (kh2 = 14.4 and p = 0.001) according to Framingham algorithm. The *us*-CRP assay revealed 28.77% of subject at moderate risk ([us-CRP] = 1-3 mg/L), 10.62% at high risk ([us-CRP-us] > 3mg/L), and 3.08% at ultra high risk ([us-CRP] > 10 mg/L).

Conclusion: Our study shows high prevalence of hypertension, obesity, metabolic syndrome and high level of *us*-CRP in health workers. These results are in line with the increasing prevalence of cardiovascular risk factors in sub-Saharan Africa. It is therefore urgent to emphases primary and secondary prevention of cardiovascular diseases in sub-Saharan countries by reinforcing health system with up skilled practitioners and by implementing policies and technical platforms in order to reduce their burden.

B-025

Importance of Quality Indicators Monitoring in Chemistry at Private Hospital, the Karen Hospital in Kenya

B. B. N. Wafula, A. Miwa, E. K. Wandia, C. Wambui, A. Kanyua. *The Karen Hospital, Nairobi, Kenya*

Background/Introduction: Quality indicators are a measure of performance in the laboratory. Any laboratory that is implementing ISO 15189:2012 clause 4.14.7 are supposed to formulate quality indicators and monitor them through all the aspect of pre-examination, examination and post examination process. The Karen Hospital is in the process of implementing the ISO 15189:2012 towards accreditation**.**

Materials and Methods: The laboratory did a baseline audit to establish the extent of conformity to the international standard which formed the basis of forming the quality indicators in September 2018. The formulation of quality indicators which included turnaround time TAT for urea , electrolytes and creatinine(UEC) , external quality assurance performance(EQA) and sample rejection rate. Initial report from the hospital information system (HIMS) INSTA was mined to get the baseline report. Following up on this, daily tracking of these parameters was recorded and a monthly tally or calculation was done.

The results were tabulated and percentages computed to check if there was improvement.

Results:

QUALITY INDICATOR	BASEINE SEPTEMBER 2018	OCTOBER 2018		NOVEMBER 2018	
		TARGET	SCORE	TARGET	SCORE
TAT(UEC)	2HRS 2 MIN-UTES	1HR 30 MINUTES	1 HR 13 MINUTES	1HR 30 MINUTES	1HR 19 MINUTES
EQA PERFORMANCE (BASIC CHEMISTRY)	86.9%	>80%	88%	>80%	88%
SAMPLE REJECTION RATE	0%	<2.5%	0%	<2.5%	0%

From the time the laboratory started monitoring the quality indicators stated a number of positive happenings were realised. First there was reduction in TAT that led to improved customer satisfaction rate, sample rejection rate was maintained at 0% and EQA performance increased to 88% from 86.9%.

Conclusion: After two months, through the daily tracking the laboratory was able to identify and eliminate bottle necks that led to shorter TAT, reduced customer complaints and the laboratory staff buy in the accreditation process.

Implementation of ISO 15189:2012 in a laboratory has great advantages. Monitoring of the quality indicators is important because it takes deliberate effort of the laboratory management to improve its services to the clients it serves and overall patient safety.

B-026

Comparison of Creatinine Measurements Between Roche Cobas C501 and Abbott Architect Ci8200 in a Routine Laboratory

P. W. KARIUKI, A. A. Amayo, I. Abdulhafedh. *THE NAIROBI HOSPITAL, NAIROBI, Kenya*

Background: Chronic kidney disease (CKD) is a global public health problem. Reliable and transferable serum creatinine measurements are important for effective management of patients with CKD. Standardization of creatinine measurements was done

by ensuring calibration traceable to the Isotope Dilution Gas Chromatography/Mass spectrometry (ID-GC/MS) method. There are however reports of instrument related biases in creatinine results which may affect transferability of results. Accredited laboratories are required to have a back-up analytical equipment for all their analytes to ensure continuity of services at all times. It is required that method comparison be done to verify that results are transferable. At the Nairobi Hospital laboratory creatinine is provided on Roche Cobas C501 and Abbott Architect Ci8200.To ensure patient safety, a method comparison study was undertaken to verify transferability of creatinine measurements.

Method: 108 paired data obtained from 2 levels of commercial internal quality control (Randox Levels 2 and 3) analyzed between June and September 2018 were used in this study. All analyses were done in accordance to manufacturer's requirements.

Statistical analysis: Quality control results were summarized using descriptive statistics (mean, standard deviation and coefficient of variation). Passing - Bablok regression analysis was used for comparison of the two methods and agreement assessed using Bland-Altman method. Student T test was used for significance testing.

Results: The mean for Randox Level 2 for Architect ci8200 was 150.1 umol/L which compared well with that for Cobas c501 (149.4 umol/l). Differences were noted between the means for Randox Level 3, being 518 umol/l and 471 umol/l for Architect ci8200 and Cobas c501 respectively (p=0.06). All the results were within the equipment specific target values provided by the IQC provider.

The coefficient of variation (CV%) for Randox level 2 was 2.4% and 2.9% for Architect ci8200 and Cobas c501 respectively. For Randox level 3 the CV % was 2.02% and 2.8 %.

The desirable specification for creatinine imprecision is 2.98%. Both equipment met these criteria.

Conclusion: In this study, both Architect ci8200 and Roche Cobas c501 were found to meet the analytical performance requirements for routine analysis of creatinine. The two methods can be used interchangeably at normal serum creatinine concentrations. The noted differences at high creatinine concentrations may affect laboratory assessment of dialysis efficacy and general monitoring of CKD patients. Additional studies, using clinical samples are required to further assess this difference. Harmonization between the two methods may be required to ensure result transferability for the higher creatinine results.

B-028

Self-Efficacy and Self-Care Behaviour and Their Relation with Glycemic Control among Nepalese Adult Patients with Type 2 Diabetes Mellitus

S. Timalsina, S. Sharma. *Chitwan Medical College, Chitwan, Nepal*

Background: Type 2 diabetes mellitus (T2DM) has become an ever-increasing burden not just to individuals but also to the health care systems worldwide. Diabetes complications, both microvascular and macrovascular can significantly inflate the cost of diabetes, particularly relevant to a developing country like Nepal. Treatment and management of diabetes is a major challenge here, for reasons such as low disease awareness among the population, various socio-cultural factors and the paucity of programs to detect, manage, and prevent diabetes and its complications. Self-efficacy (which links self-perceptions and individual actions) and self-care (the actions taken by the patients that encompass diet, exercise, medication taking (insulin or oral hypoglycemic agents), self-monitoring of blood glucose and foot care) are predictors of glycemic control and complications rate. This study was conducted to assess the status of self-efficacy and self-care behavior in Nepalese diabetic patients and their correlation with glycemic control. **Methods:** This was a cross-sectional study involving adult patients with T2DM visiting a tertiary medical care center in Chitwan, Nepal. T2DM was defined as per ADA criteria. Demographic and anthropometric variables were noted. The participants completed 2 sets of validated questionnaires: 20-item Diabetes Management Self-Efficacy Scale (DMSES) and 10-item Summary of Diabetes Self-care Activities Scale (SDSCA). Glycemic control, as represented by HbA1c levels, was categorized as good (HbA1c 6.0-7.0%), fair (7.1%-8.2%) and poor control (>8.2%). HbA1c was measured using Dimension® analyzer using turbidimetric method. **Results:** This study involved 162 patients with type 2 diabetes mellitus (Male: 80 Female: 82; mean age: 49.16 ± 9.75 years; median duration of disease: 4 years (1-25 years). 65.4% were using oral hypoglycemic agents only. 26.5% had diabetic complications present, majority having peripheral neuropathy. 50% of the patients had good glycemic control, and 14.2% had poor control. Both the used scales had good internal consistency (**Cronbach's alpha**: 0.903 and 0.741 for DMSES and SDSCA scales respectively). The mean total self-efficacy and self-care behaviour scores were 137.05 ± 24.23 and 36.75 ± 9.99 respectively, with a significant positive correlation between these 2 scores (r=0.549, P<0.001). HbA1c was negatively related with total DMSES and SDSCA scores (r=-0.18, r=-0.2 respectively, P<0.01). Multiple logistic regression analysis demonstrated higher educational level, higher total DMSES and total SDSCA scores significantly associated with good glycemic control. It was not affected by age, gender and duration of disease.

Conclusion: The DMSES and SDSCA scales are suitable for use in research and clinical settings in the Nepalese population with T2DM as measures of self-management interventions. Greater self-efficacy and self-care behavior, as given by total DMSES and SDSCA scores are strong predictors of good glycemic control, consistent across different categories of age, gender and ethnicity. Focusing on improvement in self-care behaviors can be one of the important cost-effective interventions in minimization of complications and improvement of the quality of life of these patients.

B-029

Assessment of the Quality Control Strategy of HbA1c by Risk Management Index

H. Hengjian, M. Liye, P. Shiyun, Y. Pei, Z. Li. *West China Hospital of Sichuan University, Chengdu, China*

Background: Risk management index (RMI) was used to evaluate different quality control (QC) strategies for laboratory HbA1c analyzers.

Method: RMI = expected P_H / acceptable P_H, where (P_H is the probability of patient harm). Expected $P_H(SE) = [P_E(0) + E(N_{uf}(SE))/MPBF]*P_{hlu}$. $P_E(0)$ is the probability of unacceptable results by the glycated hemoglobin analyzer in control. $E(N_{uf}(SE)$ is the expected number of final unacceptable reports for the device under systematic error. MPBF is the mean number of tests between two neighboring out-of-control intervals, which is the number of samples measured every day multiplied by the mean number of days between out-of-control intervals. P_{hlu} is the probability of unacceptable test results causing harm to the patients. $P_E(0)$ and $E(N_{uf})$ were calculated using the standard normal cumulative distribution function. The acceptable P_H was obtained from the CLSI EP23-A acceptable risk model and ISO 14971 guideline matrix. A series of QC strategies was designed for laboratory glycated hemoglobin analyzers which test 300 samples every day: The analytical run length was defined as 50, 100, 150, 200, 300, and QC rules 1_{3s} N1, 1_{3s} N2, $1_{2.5s}$ N1, $1_{2.5s}$ N2, 1_{2s} N1, and 1_{2s} N2 were used. The mean number of days between out-of-control intervals was 30 days.

Results: $P_E(0) = 6.74*10^{-5}$, MPBF = 300*30 = 9000; $P_{hlu} = 0.50$; acceptable $P_H = 1.00*10^{-4}$. Analytical run lengths were 50, 100, 150, 200, and 300. The $E(_{Nuf})$ of the QC rule 1_{3s} N1 was 0.57, 1.14, 1.71, 2.27, and 3.41, respectively; the expected P_H was $6.53*10^{-5}$, $9.69*10^{-5}$, $1.29*10^{-4}$, $1.60*10^{-4}$, and $2.23*10^{-4}$, respectively; and RMI was 0.65, 0.97, 1.29, 1.60, and 2.23, respectively. For QC rule 1_{3s} N2, the $E(_{Nuf})$ was 0.12, 0.24, 0.37, 0.49, and 0.73, respectively; the expected P_H was $4.05*10^{-5}$, $4.73*10^{-5}$, $5.41*10^{-5}$, $6.08*10^{-5}$, and $7.44*10^{-5}$, respectively; and RMI was 0.41, 0.47, 0.54, 0.61, and 0.74, respectively. For QC rule $1_{2.5s}$ N1, the $E(_{Nuf})$ was 0.23, 0.45 , 0.68, 0.90, and 1.36, respectively; the expected P_H was $4.63*10^{-5}$, $5.88*10^{-5}$, $7.14*10^{-5}$, $8.39*10^{-5}$, and $1.09*10^{-4}$, respectively; and RMI was 0.46, 0.59, 0.71, 0.84, and 1.09, respectively. For QC rule $1_{2.5s}$ N2, the $E(_{Nuf})$ was 0.04, 0.07, 0.11, 0.15, and 0.22, respectively; the expected P_H was $3.58*10^{-5}$, $3.78*10^{-5}$, $3.99*10^{-5}$, $4.20*10^{-5}$, and $4.61*10^{-5}$, respectively; and RMI was 0.36, 0.38, 0.40, 0.42, and 0.46, respectively. For QC rule 1_{2s} N1, the $E(_{Nuf})$ was 0.08, 0.17, 0.25, 0.33, and 0.50, respectively; the expected P_H was $3.83*10^{-5}$, $4.29*10^{-5}$, $4.75*10^{-5}$, $5.22*10^{-5}$, and $6.14*10^{-5}$, respectively; RMI was 0.38, 0.43, 0.48, 0.52, and 0.61, respectively. For QC rule 1_{2s} N2, the $E(_{Nuf})$ was 0.01, 0.02, 0.03, 0.04, and 0.06, respectively; the expected P_H was $3.43*10^{-5}$, $3.48*10^{-5}$, $3.53*10^{-5}$, $3.58*10^{-5}$, and $3.68*10^{-5}$, respectively; and RMI was 0.34, 0.35, 0.35, 0.36, and 0.37, respectively.

Conclusion: An RMI ≤ 1.00 shows that the QC strategy for glycated hemoglobin analyzers can control patient risk to an acceptable range. Our study selected the QC strategy of 1_{3s} N1 and analytical run length of 100 or $1_{2.5s}$ N1 and an analytical run length of 200 to test 300 glycated hemoglobin samples every day.

B-030

Evaluating the Components of Risk of Patient Harm from Erroneous Results

J. C. Yundt-Pacheco. *Bio-Rad Laboratories, Plano, TX*

Background: Using a risk-based approach to QC requires estimating the risk of patient harm from erroneous results (in this case using a Risk Management Index-RMI), after which, a lab must determine what to do if there is an unacceptable level of risk (RMI>1)?

To correct the situation, one must assess the components of risk. What part of the overall risk can be attributed to in-control imprecision, in-control bias, or out-of-control conditions? If too many erroneous results are produced because of in-control imprecision, there is no QC strategy that will fix it. A similar argument can be made for bias. While the production of erroneous results from out-of-control conditions is not independent of in-control performance, it is still useful to assess how much risk is attributed to out-of-control conditions which may be mitigated by adjusting the QC strategy. The method below evaluates these risk components.

Methods: The sources of risk of patient harm from erroneous results are computed as:

$P_E(0)$[probability of in-control erroneous results]=f(x)[1-Φ([x+TE_a(x)-bias(x)]/σ(x))+Φ([x-TE_a(x)-bias(x)]/σ(x))]

$P_{Ei}(0)$[probability of erroneous results from imprecision]=$P_E(0)$-f(x)[1-Φ([x+TE_a(x)]/σ(x))+Φ([x-TE_a(x)]/σ(x))]

$P_{Eb}(0)$[probability of erroneous results from bias]=$P_E(0)$-$P_{Ei}(0)$

$P_{\Delta E}$[probability of out-of-control erroneous results] =∫[E(Nuf(SE))/[MPBF+ANPed(SE)] *dSE* over ±2 TE_a range.

P_E[probability of erroneous result]=$P_{Ei}(0)$+$P_{Eb}(0)$+$P_{\Delta E}$

P_{PH}[predicted probability of harm]=P_E*$P_{h|u}$

Acceptable P_H[acceptable probability of harm] is derived from Severity of Harm and risk acceptability matrix.

RMI (RMI<=1 indicates managed risk)=P_{PH}/Acceptable P_H

Formulas, terms and definitions are abbreviated for abstract.

Results: Computation Example

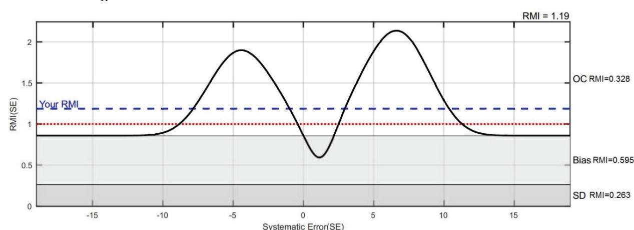
Glucose:CV=2.5%,TE_a=±10%,bias=+1.

QC Strategy:2QC levels,1:3 Rule, every 50 results.

MPBF=9,000.

$P_{h|u}$=0.5.

Acceptable P_H=1/10,000.



Conclusions: Breaking the RMI of a test method into the individual components of risk gives the laboratorian critical information for resolving risk issues - is the problem imprecision, bias or the quality control strategy, or some combination? Once the risk components have been evaluated, steps can be taken to mitigate risk by reducing the risk associated with bias, or imprecision, or the quality control strategy.

B-031

Imprecision Analysis of Internal Quality Control of Six Sex Hormones Tests in Clinical Laboratories in China from 2016 to 2018

H. Sun, W. Wang, F. He, Y. Du, S. Yuan, J. Liu, Z. Zhang, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

Background: Six sex hormones, including estradiol, follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, progesterone and testosterone are commonly measured clinically to understand endocrine function and diagnose endocrine diseases. This study aimed to investigate and analyze the overall imprecision of internal quality control (IQC) for these sex hormones in China from 2016 to 2018.

Methods: Three years' cumulative coeffcient of variation (CV) and other IQC information of these analytes were collected via the Clinet-EQA reporting system established by the National Center for Clinical Laboratories in China. The percentages of laboratories meeting the quality requirements based on biological variation, including minimum, desirable and optimal specification were calculated (i.e., pass rate). Friedman M test was performed in each analyte to compare the cumulative CVs among different years.

Results: From 2016 to 2018, there were 866, 773, 868, 865, 872 and 858 laboratories continuously submitting their IQC data for estradiol, FSH, LH, prolactin, progesterone and testosterone, respectively. The results are shown in the table below. For analytes except FSH and progesterone, there were significant differences in cumulative CVs among 3 years (all $P<0.01$). Further analysis showed that significant differences of cumulative CVs for estradiol and prolactin were between the following 2 years: 2016 and 2018, 2016 and 2017; for LH were between 2016 and 2018; for testosterone were between 2016 and 2018, 2017 and 2018 (all $P<0.03$). The percentages of laboratories meeting minimum specification were satisfied (pass rates>88%) for analytes except testosterone. While applying the optimal specification, only a few laboratories can meet the requirement (pass rates<70%).

Conclusions: The imprecision for each analyte except testosterone was significantly improved in China from 2016 to 2018. However, laboratories should continue working hard to meet the optimal specification especially for testosterone. Laboratories are recommended to select more appropriate quality specifications to evaluate their analysis performance.

Item	Year	Cumulative CVs (%)		Percentages of laboratories meeting quality requirements %(N)		
		Median	IQR	Minimum specification	Desirable specification	Optimal specification
Estradiol	2016	6.87	4.82	95.84 (830)	85.80 (743)	36.72 (318)
	2017	6.59	5.00	95.96 (831)	85.45 (740)	40.07 (347)
	2018	6.42	4.75	96.30 (834)	87.41 (757)	41.69 (361)
FSH	2016	4.92	2.85	94.57 (731)	61.32 (474)	13.71 (106)
	2017	4.96	3.36	88.62 (685)	58.73 (454)	15.01 (116)
	2018	4.90	3.10	89.52 (692)	60.93 (471)	16.56 (128)
LH	2016	5.53	3.31	99.31 (862)	96.77 (840)	53.23 (462)
	2017	5.36	3.33	99.42 (863)	97.24 (844)	53.80 (467)
	2018	5.22	3.31	99.78 (866)	96.89 (841)	57.49 (499)
Prolactin	2016	5.02	3.27	99.54 (861)	97.57 (844)	60.92 (527)
	2017	4.97	2.95	99.65 (862)	98.15 (849)	65.43 (566)
	2018	4.66	3.04	99.77 (863)	98.15 (849)	66.82 (578)
Progesterone	2016	6.78	4.66	91.40 (797)	77.52 (676)	26.72 (233)
	2017	6.74	4.45	93.12 (812)	78.56 (685)	27.06 (236)
	2018	6.64	4.24	92.78 (809)	81.08 (707)	29.13 (254)
Testosterone	2016	5.81	4.19	64.69 (555)	33.80 (290)	4.08 (35)
	2017	5.60	4.03	64.10 (550)	34.73 (298)	4.90 (42)
	2018	5.26	3.47	70.63 (606)	39.39 (338)	6.18 (53)

B-032

Status of Critical Values Notification in 2931 Clinical Laboratories in China from 2015 to 2018

H. Sun, W. Wang, Y. Du, J. Liu, M. Duan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

Background: In the era of patient-centered medicine, laboratory medicine should pay more attention to patients and their safety. Critical values are the values that must be reported to the clinic when they appear. Whether critical values are reported or not and timeliness of notification can directly affect patients safety. The percentage of critical values notification and percentage of timely critical values notification are two important quality indicators (QIs) in post-analytical phase, which can reflect the situation of critical values notification. This study aimed to investigate the status of these two QIs in clinical laboratories in China from 2015 to 2018.

Methods: The National Center for Clinical Laboratory (NCCL) in China has conducted annual national surveys of QIs from 2015 to 2018. Participant laboratories were required to submit their results via network platform. The percentage of critical values notification and percentage of timely critical values notification were evaluated

by percentage and sigma metrics (σ). Friedman M test was adopted to perform the comparison among different years.

Results: From 2015 to 2018, there were 2931 laboratories continuously submitting their data of these two QIs. 2256 (76.97%) participant laboratories notified all critical values among 4 years, and 1875 (63.97%) laboratories could notify their critical values timely for 4 consecutive years. When using percentage, the median of both QIs were 100.00% and were both 6.00 when σ metrics was used. The 4 years' results are shown in the table below. There were significant differences in the percentage of timely critical values notification between 2015 and 2016 (P=0.047).

Conclusion: The majority of participant laboratories could report all critical values timely among 4 years. However, the situation of critical values notification in some laboratories still needs to be improved. Clinical laboratories are supposed to focus on critical values notification continuously, so as to ensure patients safety.

Year	Percentages of critical values notification				Percentages of timely critical values notification			
	Mean (%)	Median (%)	Mean (σ)	Median (σ)	Mean (%)	Median (%)	Mean (σ)	Median (σ)
2015	98.37	100.00	5.71	6.00	94.76	100.00	5.36	6.00
2016	98.96	100.00	5.76	6.00	98.83	100.00	5.60	6.00
2017	98.23	100.00	5.71	6.00	97.95	100.00	5.55	6.00
2018	98.32	100.00	5,70	6.00	97.70	100.00	5.53	6.00

B-033

Improving Clinical Toxicology Utilization for Independent Practices through Data Analysis and Educational Tools

J. P. Canner. *Gravity Diagnostics, Covington, KY*

Background: Best practices of clinical toxicology recommends individual locations determine which drugs/analytes to examine based on their patient population. Oftentimes, smaller independent practices are not aware of the changing drug patterns within their area or among their patient population. In addition, reference laboratories build their test menus to encompass the greatest number of drugs/analytes to cover diverse clients and a national patient population. These two factors result in over-utilization of clinical toxicology testing with providers often ordering various drug classes due to their availability rather than necessity. Analysis of individual practice's data allowed for the creation of a new test requisition form and physician educational tools to aid with clinical toxicology ordering.

Methods: One pain management location's 2018 clinical toxicology data was mined to determine what effects the new test requisition form and educational tool had on their ordering pattern and detection of unexpected substances. The location's ordering patterns were determined by analyzing the number of toxicology CPT codes (80307, G0480 - G0483) billed by month. 2379 samples from 290 patients were tested between January 2018 and December 2018. The rate of drugs/analytes with inconsistent positive detection for each month was calculated and the top drugs were analyzed over the course of the year. Cost analysis utilized the ordering pattern by month with the 2018 CMS Clinical Laboratory Fee Schedule to determine the potential financial impact of these tools.

Results: Analysis of the billing data showed every sample had an immunoassay screen preformed (80307). In January 2018, 234 samples were processed with a tier 4 (G0483) order. July 2018 was the month before the new requisition form and education tool was utilized and there were 2 tier 3 (G0482) and 180 tier 4 (G0483) orders. September 2018 was the month after implementation and 6 tier 1 (G0480), 164 tier 2 (G0481), 2 tier 3 (G0482), and 3 tier 4 (G0483) were ordered. In December 2018, 30 tier 1 (G0480), 9 tier 2 (G0481), 151 tier 3 (G0482), and 0 tier 4 (G0483) were ordered. Morphine, oxazepam, and amphetamine had some of the highest inconsistent positive detection rates. No differences in the detection rate was observed for these drugs over 2018. Applying the 2018 CMS Clinical Laboratory Fee Schedule to the ordering pattern, we see a $63.49 average reduction per sample. The average number of times a patient was tested over 2018 was 8.2, which would be a $520.61 reduction per patient. In total, by changing the ordering pattern at this pain management location, the 20% reduction in per sample order would reduce the practice's billing charges by $151,309.58.

Conclusion: We developed a new test requisition form and educational tool to improve the ordering patterns of independent practices. These tools were able to reduce the billing charges by 20% per sample which would result in one practice saving $151,309.58 in healthcare costs. In addition, we were able to show several drugs with high illicit use were still detected at similar rates before and after the change in ordering behavior.

B-034

Validation Verification of Creatinoquinase (CK-L) Reagent in the ADVIA 1800 Siemens™ System

M. Soika, D. Faria, D. Canali, C. Vieira, A. Lopes, M. Valente. *Laboratório Médico Santa Luzia, São José, Brazil*

Background: The reagents involving enzymatic reactions have been undergoing upgrades to improve stability and calibrations, including traceable standards. The manufacture Siemens has upgraded its Creatine Kinase reagent (CK-L) to the ADVIA Chemistry System regarding future discontinuation of the current reagent (CKNAC). The improvements involve: liquid and ready-to-use reagents, higher on board stability (from 10 to 30 days) and specific calibrator compared to the International Federation of Clinical Chemistry (IFCC) method. This study is aimed to verify the quality requirements for new reagent compared to the CKNAC.

Methods: Performance characteristics were tested according to laboratory operating procedures, based on Clinical & Laboratory Standards Institute (CLSI) recommendations (EP5-A3, EP9-A3, EP10-A3, EP6-A and EP-15-A3). The following parameters were evaluated: Imprecision (2 samples: 36 and 245 U/L, processed 5 times a day for 5 days), Accuracy (comparison between 20 samples processed between new reagents (CK-L) and the old one (CKNAC), Linearity (5 consecutive dilutions from the most concentrated sample) and Carryover (3 low, intermediate and high samples, intercalated). All blood samples were processed in ADVIA 1800 System™ Siemens.

Results: The Imprecision intra-day were 1.12% and 0.60%, while the inter-day values were 1.52% and 0.48% for the low and high levels, respectively. The coefficient of variation (CV) obtained was lower than defined by the manufacturer (4.5%). In the accuracy analysis, the regression analysis ($r2$) was 0.999 (acceptable ≥0.975) and the error index analysis resulted in 100% acceptable. The method was linear between concentrations 16 and 4986 U/L and no carriage was evidenced between the samples.

Conclusion: The results showed excellent performance to reformulated CK-L reagent. Equivalent to the CKNAC reagent.

References: 1. Clinical and Laboratory Standards Institute. Evaluation of Precision Performance of Quantitative Measurement Methods. Wayne: USA; 2014, 3rd edition, CLSI Document EP5-A3.

2. Clinical and Laboratory Standards Institute. Measurement Procedure Comparison and Bias Estimation Using Patients Samples. Wayne: USA; 2013, 3rd edition, CLSI Document EP9-A3.

3. Clinical and Laboratory Standards Institute. Preliminary Evaluation of Quantitative Clinical Laboratory Measurement Procedures. Wayne: USA; 2014, 3rd edition, CLSI Document EP10-A3.

4. National Committee for Clinical Laboratory Standards. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. Wayne: USA; 2003 NCCLS Document EP6-A.

5. Siemens Healthcare Diagnostics Inc., Datasheet CK_L - 10720946_PTBR. 2018 Review B, 2018-03.

6. Siemens Healthcare Diagnostics Inc., Datasheet CKNAC - 10699615_PT. 2017 Review F, 2017-03.

B-035

Application of Lean Six Sigma and Computer-Aided Simulation Methodologies to Improve Turnaround Time of Blood Transfusions

J. A. Baena-Del Valle, A. Alvarado-Trujillo, C. Marin, M. Mora, A. Torres, M. Daza, M. Silgado, M. A. Paez-Cancelado. *Fundacion Santa Fe De Bogota, Bogota, Colombia*

Background: Turnaround time (TAT) is the time between the physician order for transfusion and the beginning of the infusion; it is an important indicator of patient outcome and medical customer satisfaction. According to the Annual Shot Report 2017, delays are the second cause of deaths related to transfusions. The aim of this study was to improve the TAT by using cost-effective methodologies in a tertiary care hospital in Bogotá, Colombia.

Methods: TAT (and time between all pre-transfusion procedures) from 1626 transfusions were recorded during a two-year period (2016 - 2017). Descriptive statistics, frequency distributions, and comparison tests were applied. A Lean Six Sigma approach was performed by 1) Defining the problem baseline 2) Defining the scope of the project 3) Creating the work team 4) Creating a critical to quality tree 5) Using tools such as process flowchart, process analysis, function deployment matrix, relationships diagram, and work plan. The previously recorded time data was used to recreate the current transfusion process and some improved alternatives generated from the Lean

Six Sigma on a Computer-Aided Simulation via ProModel v.9.3.1.2081. Five simulation runs of one month each were performed. The process with the best performance was chosen and a pilot test was conducted.

Results: The mean TAT was 9 hours and 21 minutes and a median of 5 hours and 6 minutes, it did not change significantly ($p = 0.5493$) across the main blood components, number of units issued ($p = 0.8234$) or time of the day when the transfusion was requested ($p = 0.3069$). The longest pre-transfusion procedures were: physician's request-to-blood draw time (1:43:16) and pre-transfusion testing sign-out-to-blood issue (4:32:28), since these were entirely dependent on the availability of floor nurses and patient/sample transporters to start the pre-transfusion procedures and collect the blood components for infusion, respectively. By applying Lean Six Sigma strategies, we generated simplified flowcharts on pre-transfusion procedures, that features real-time monitoring of transfusion requests at the blood transfusion laboratory, hiring a nurse/lab aid to expedite blood draw for pre-transfusion testing and ensure on-time dispatch of infusion sets. Simulation runs helped select the most cost-effective process (improvement in the in-operation percentage from 35% to 60%, and reducing TAT to 3 hours). A pilot test was run for 2 months (241 transfusions) and showed similar TAT to the one obtained by simulation (3.22 hours). The changes were finally approved by the hospital board.

Conclusion: Process improvement methodologies (e.g. Lean Six Sigma) are useful to improve a number of processes in health care, some of them critical for their implications in mortality, as the timeliness of blood transfusion. We frequently face various alternatives for improvement, and that is where computer simulation tools help us evaluate these new ideas before using time and expensive resources to apply modifications; They also allow us adding many complex scenarios and operational strategies to achieve the best results for the benefit of the patient and the client.

B-036

Improving Thyroid Function Test Utilization via Implementation of a Reflexive Testing Algorithm

E. Garnett[1], J. Pagaduan[1], S. Mehravaran[1], M. Wong[1], R. Huang[2], J. Cao[3], S. Devaraj[3]. [1]*Baylor College of Medicine, Houston, TX,* [2]*Houston Methodist Hospital, Houston, TX,* [3]*Baylor College of Medicine/Texas Children's Hospital, Houston, TX*

Background: Thyroid function test utilization is problematic, as the wide menu of available thyroid tests allows clinicians to potentially order obsolete, redundant, or otherwise unnecessary tests. To address this issue, the American Thyroid Association, American Association of Clinical Endocrinology, and National Academy of Clinical Biochemistry all recommend a screening algorithm for detection of thyroid function disorders. Our institution is implementing such an algorithm in 2019, recommending thyroid-stimulating hormone (TSH) with reflex to free thyroxine (FT4). This study sought to evaluate the potential of thyroid function testing algorithms to reduce inappropriate test usage and reduce overall thyroid testing burden in our hospital.

Methods: A retrospective analysis was conducted by chart review of 50 patient records for whom thyroid function tests (TSH, FT4, total T4, FT4 by dialysis, total T3, free T3, T3 uptake, and reverse T3) had been requested in the month of October 2018 at Texas Children's Hospital. For each record, the requested thyroid tests were evaluated in context of the new algorithms (for endocrinology or hematology/oncology, TSH and FT4 may be ordered together; for all other specialties, TSH is ordered with reflex to FT4) to determine whether each order would have been excluded based on testing guidelines. Thyroid function testing practices for the entire institution over the past 12 months (January 2018-January 2019) were evaluated using EPIC SlicerDicer.

Results: Of a total of 171 tests in one month, we found that 80 test orders (47%) would have been avoided by following the algorithm, with FT4 orders reduced by 46%, total T4 orders by 67%, and total T3 by 88%. Free T3, reverse T3, and T3 uptake were not indicated by the algorithm. For ordering physicians not in endocrine or hematology/oncology specialties, impact was greater: out of 111 tests, 68 (61%) would have been avoided by following the algorithm, with utilization of FT4 reduced by 67%, total T4 by 87%, and total T3 by 100%. Only 3 of 50 (6%) of patients had abnormal FT4 or total T4 with TSH within the reference interval, which would have been missed by our algorithm. Over the previous year at our institution, 67,407 thyroid function tests were ordered. Based on expected percent reduction per test, 17,340 (26%) orders would be avoided through adherence to the testing algorithm.

Conclusion: This study indicates that adherence to recommended testing algorithms would substantially reduce inappropriate thyroid function test orders. This study is also consistent with previous work at our institution, which found that 96% of thyroid function disorders are appropriately identified by our new thyroid function testing algorithms, even in patients with comorbidities. Together, these findings indicate that implementation of thyroid function screening algorithms would result in improved test utilization and efficient patient care.

B-037

Defining Quality Goals: Navigating with GPS

G. Pacheco, M. N. Zubillaga, M. E. Olivera, M. Risso, M. H. Fornella, B. Varela. *SIEMBRASUR, Montevideo, Uruguay*

Objective: The purpose of this work is to evaluate the new selection algorithm for a Total Allowable Error (TEa) source using a renewed graphic tool which, by integrating Internal (IQC) and External (EQC) Quality Control performances, enables the laboratory to evaluate the TEa source that better fits the test analytical performance, as well as to evaluate the analytical system stability throughout the period examined. It is worth noting that this new algorithm is based on the previous algorithm model published by the laboratory on Biochemia Medica journal in June 2018 (*https://doi.org/10.11613/BM.2018.020710*). **Materials and Methods:** The results of surveys of the External Quality Evaluation Program EQAS® BIO-RAD from 2018 were used to evaluate the performance of 23 biochemistry tests. The evaluated assays were processed in a homogenous system, Abbott manufactured analytical platform Architect ci8200. Two analytical performance indicators (sigma metric and bias) were estimated for each test following the sequence step algorithm**.** Sigma metric was calculated through the results obtained in the IQC, and the Bias was estimated based on the EQC program. The sigma metric was charted as a function of the bias expressed as the percentage of the TEa [Bias (%TEa)]. The algorithm is divided into 3 steps: 1) Selection Step (SS), 2) Evaluation Step (ES) and 3) Decision Step (DS). In the SS, using the 2017 data and following the proposed algorithm (considering the hierarchy in the Milan 2014 Consensus & the Government Regulations of each country), the TEa was evaluated depending on 2 areas. One area in the chart was defined as the objective area ($6 < \text{Sigma} < 12$ and Bias (%TEa) < 45 or Sigma >12 and $25 <$ Bias (%TEa) < 45) in which the used TEa is the appropriate one for the analytical performance of the test under evaluation. For every test located outside the objective area (second area), a performance reevaluation was required using another TEa source. In the ES, using the 2018 data, performance indicators were calculated using the TEa selected in the SS. In the DS, tests which presented a change in location within the chart (ES in relation to SS) were identified to analyze whether ETa redefinition was necessary. **Results:** A total of 23 biochemistry tests were evaluated. Selection Step: In 19 of the evaluated tests, the resulting analytical performance allowed for the selection of biologic variability as TEa source. In the 4 remaining cases (Calcium, Magnesium, Total Protein and Sodium), State of the Art (Rilibak) was selected. Evaluation Step: Only one of the evaluated tests (LDH) presented a change in location in relation to 2017. Decision Step: ETa redefinition did not apply for the case problem detected (LDH). **Conclusions:** The new TEa selection algorithm made possible its adaptation to a regulatory framework on TEa usage. Furthermore, the redefinition of the objective area enabled its use as a tool for the evaluation of the analytical system stability throughout time.

B-038

Evidence-Based Laboratory Quality Improvement: A6 Cycle and Guideline Development

L. Williams. *CDC, Atlanta, GA*

Objectives: An initial step in developing evidence-based laboratory guidelines is a systematic review of the evidence for laboratory practices. The Centers for Disease Control and Prevention Division of Laboratory Systems (DLS) supports the Laboratory Medicine Best Practices (LMBP) initiative, with a vision of evidence-based laboratory medicine quality improvement in support of health care and patient outcomes. This is achieved through the A6 cycle method for systematic reviews. The A6 cycle is a multidisciplinary, six-step process that serves to develop (steps A1 - A4), as well as evaluate (steps A5-A6), evidence-based laboratory medicine best practices. Key to success is production of evidence-based best practices with practical utility to laboratory professionals. Laboratory-centric modifications distinguishing the A6 cycle method from other methods are: 1) inclusion of quality improvement studies, 2) inclusion of unpublished evidence, 3) combining of individual ratings of study quality and effect size to evaluate consistency of evidence, and 4) deriving an overall strength of evidence rating. The LMBP initiative and the A6 cycle are uniquely framed in relation to the National Academy of Medicine's six domains of health care quality.

Methods: Systematic reviews were performed with external consultants, subject-matter experts, and professional organizations (e.g., the American Society for Microbiology and the American Association for Clinical Chemistry). The collaborative process involved identification and prioritization of nationally important laboratory medicine

quality gaps. The process then involved "Asking" a focused research question, "Acquiring" the relevant evidence base of quality improvement studies, "Appraising" the quality and effect sizes of the studies, and "Analyzing" the evidence through criteria-based grading of the overall strength of the body-of-evidence.

Results: Since 2012, eleven systematic reviews have been published and two reviews are currently accepted for publication. Four reviews are in progress, while four reviews previously published are being updated. Examples include reviews to assess the effectiveness of practices to reduce rates of blood culture contamination, assess the use of nontraditional lipid biomarkers, improve timely diagnosis of blood stream infections, reduce over- and under-utilization of testing, and reduce rates of tests pending at discharge.

Conclusion: The LMBP A6 cycle has produced systematic reviews with evidence-based findings that have practical utility to clinical laboratory professionals, and have the potential to strengthen laboratory services. As best practices are identified, evidence-based laboratory guidelines can be developed through partnering with professional organizations and stakeholders. To better achieve success, additional networks and stakeholder involvement are sought.

B-039

Cooled Storage Space and Solid Infectious Waste Production: Results of a Comparative Study across Six Immunochemistry Analysers

P. Findeisen[1], I. Zahn[1], D. Krempel[1], C. Garcia Rabaneda[2], M. T. Haro Romero[2], T. De Haro-Muñoz[2], M. Barral Juez[2], S. Engelmann[3]. *[1]Laboratory Dr. Limbach and Colleagues, Heidelberg, Germany, [2]UGC Análisis Clínicos intercentros, Hospital Universitario Campus de la Salud, Granada, Spain, [3]Roche Diagnostics GmbH, Mannheim, Germany*

Background: During this study, we analyzed two aspects - reagent- and waste handling - that are of high relevance for everyday organization in diagnostic laboratories. We compared six commercially available immunochemistry analysers, with respect to cooled space required to store reagents and their production of solid infectious waste.

Methods: The comparative evaluation was performed at two laboratories in Germany and Spain on analyzers from five different manufacturers: two **cobas e** 801 analytical modules (Roche Diagnostics), ARCHITECT i2000SR (Abbott), UniCel DxI 800 (Beckman Coulter), Liaison® XL (DiaSorin), ADVIA Centaur XPT and IMMULITE 2000 XP*i* (Siemens Healthineers). Demands for cooled storage space were determined for typical clinical laboratory assays on basis of the respective manufacturer's reagent packaging. Our analysis included a total of 18 assays (Ferritin, PTH, Estradiol, FSH, beta-HCG, LH, Progesterone, Prolactin, Testosterone, AFP, CA 125, CA 15-3, CA 19-9, CEA, fPSA, tPSA, free T4, TSH) covering reagents from five indication areas. The production of potentially infectious solid waste was evaluated by processing standardized hospital /commercial laboratory like workloads on each analyser and determining the weight of the solid waste output. In addition, the solid waste production per determination including that contributed by the empty reagent packs was calculated per system.

Results: The cooled storage space requirement for the test panel differed significantly among the included analysers. Total storage volumes (L) ranged from 79L (**cobas e** 801 system) up to 925L (Immulite 2000 XP*i*) for an identical number of determinations including calibrators. The amount of potentially infectious solid waste ranged between 0.6g (**cobas e** 801 system) per determination up to 2.8g (ADVIA Centaur XPT). Considering the solid waste contributed by empty reagent packs per determination and identical workloads representing both commercial and hospital like workloads, a waste reduction of up to 77.5% was observed on the **cobas e** 801 system in comparison to ADVIA Centaur XPT.

Conclusion: Our study provides laboratory managers with comparative cooled storage space and solid waste data over various commercially available immunochemistry systems.

B-040

How to Qualify a Reference Laboratory

V. T. Alastico, M. M. Oliveira, V. A. Alves, C. Rossin. *DASA - Diagnostico da America, Barueri - SP, Brazil*

Background: The segment of diagnostic medicine has been growing gradually in the recent years. And with this, it increases the search for partnerships with other laboratories that can offer the most advanced diagnostic solutions due to scientific and technological advances. In Brazil, the competitiveness of the market has brought a significant change in the view of clients, patients and physicians, providing a wide choice for services of high complexity with better terms, focus on quality, high knowledge and cost / benefit. In this study, we used the PDCA tool (Plan, Do, Check and Action) to create indicators to evaluate the performance and level of service of reference laboratories. Purpose: To monitor the performance of support laboratories during the period of 2017 and 2018, through indicators.

Methods: We performed a statistical study in the period of 24 months, in which we defined five parameters of analysis for the qualification and maintenance of reference laboratories. For this reason, we defined targets for three of the indicators, for compliance with deadlines (94%), for resubmit (1.5%) and for rectification of reports (0.01%). In addition, we evaluated the aspects related to the logistics chain and the service. Furthermore, the monitoring of these parameters were monthly by using graphs for each partner laboratory.

Results: During the year 2017, we outsourced 74.743 examinations tests. In addition, 4.689 delays in the release of reports, that is, 93% of TAT's deadline. Additionally, the other indicators had performed in the target. From this, we identify the main causes as incorrect test periods, lack of training of the team that prepares and sends samples, improper registration and failure in the existing process flow.

Conclusion: In the year of 2018, we achieve the deadline, presenting a TAT of 99%, which is an improvement over the previous year and exceeding our target. The indicator of resubmit and rectification of report remains in conformity with the goal. The complexity, low frequency, and extensive menu of exams challenge us to seek improvements in the rectification indicators of reports and new collections. In regards of rectification of reports, the challenge is the implantation of information technology tools between the systems and that allow identification of non-conformity before the release for patients. Moreover, the challenge of the resubmits had its origin in the qualification of personnel of collection and linked to the transport of the samples.

B-041

Imprecision Investigation and Analysis of Internal Quality Control of Hemoglobin A2 Test in Clinical Laboratories in China from 2016 to 2018

S. He, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

Background: Hemoglobin A2 (HbA2) was important in the diagnosis of thalassemia. This study observed and analyzed the internal quality control of hemoglobin A2 from 2016 to 2018 so that we could have an overall knowledge about imprecision level in Chinese clinical laboratories.

Methods: The internal quality control data including cumulative coefficient of variation (CVs) was collected with the help of the software developed by National Center for Clinical Laboratories (NCCL). The percentages of laboratories meeting quality requirements were obtained by comparing cumulative CVs with quality specification based on 1/3 TEa (6.67%) and 1/4 TEa (5.00%) defined by NCCL in China. All statistical analyses were accomplished by SPSS (IBM SPSS Statistics 21) and Microsoft Office Excel. Chi-square (χ^2) test was used to compare the percentages of laboratories meeting quality requirements among different years.

Results: There were 104 laboratories continuously submitting their imprecision data of hemoglobin A2 from 2016 to 2018. We could see from the table below that the median of CVs for HbA2 tended to decline with the passage of time and percentages of laboratories meeting quality requirements were more than 80% when 1/3TEa criteria was applied. When compared with 1/4TEa quality requirement, the percentages of qualified laboratories dropped a little, but still above 70%. The results of χ^2 test showed that there was no significant difference in percentages of laboratories among different years no matter which criteria was used (P=0.600, 0.149).

Conclusions: To sum up, internal quality control practice of hemoglobin A2 was satisfactory in Chinese laboratories from 2016 to 2018 when 1/3 TEa and 1/4 TEa quality requirements were applied, but there was still a fraction of laboratories which could not meet these requirements. These clinical laboratories should do their best to find the defects in their internal quality control practice and correct them to reach a better quality level.

Analyte	The number of laboratories	Years	Cumulative CVs (%)		Percentages of laboratories meeting quality requirements (%)	
			Median	IQR	1/3TEa(%)	1/4TEa(%)
HbA2	104	2016	3.53	2.43	90.38(94/104)	75.00(78/104)
		2017	3.51	2.33	89.42(93/104)	77.88(81/104)
		2018	2.95	1.59	93.27(97/104)	85.58(89/104)

B-042

National Survey on Pre-Examination Turnaround Time of Clinical Laboratories in China from 2016 to 2018

S. He, W. Wang, F. He, K. Zhong, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, Beijing Hospital, National Center of Gerontology, Beijing, China*

Background: Turnaround time was one of the most valuable quality indicators, which was the foundation of the quality of clinical laboratories and could affect the timeliness of providing test results. This study investigated the pre-examination turnaround time (TAT) of hospitalized tests for biochemistry, immunology and coagulation from 2016 to 2018 to observe the changes of pre-examination TAT.

Methods: The medians of pre-examination TAT for biochemistry, immunology and coagulation form 2016 to 2018 were collected with Clinet-EQA software developed by National Center for Clinical Laboratories (NCCL) in China. The 5th percentile (P5), 25th percentile (P25), Median, 75th percentile (P75) and 95th percentile (P95) of the medians of pre-examination turnaround time were calculated. Friedman M test was used to compare pre-examination TAT among different years and multiple comparisons were realized by Bonferroni method.

Results: There were 4775, 4172 and 4552 clinical laboratories continuously taking part in this survey and submitting the results of pre-examination TAT for biochemistry, immunology and coagulation from 2016 to 2018. As shown in the following table, the medians of pre-examination turnaround time were 40 to 60 minutes for these three disciplines. For biochemistry and coagulation, there were no significant difference among different years (P=0.224 and 0.206). Although the P value of immunology was 0.017, multiple comparisons did not show any significant difference.

Conclusions: Most clinical laboratories in China could ensure that specimens would be transported to their laboratories within 120 minutes, which was of important meaning for the accuracy of test results and the timeliness of test reports. Many clinicians would judge the quality of clinical laboratories based on turnaround time which was also the main source of complaints. Therefore, much attention should be paid on shortening turnaround time to provide better service for clinicians and patients.

Disciplines	The number of laboratories	Years	Pre-examination turnaround time (min)				
			P5	P25	Median	P75	P95
Biochemistry	4775	2016	10	30	57	70	120
		2017	10	30	55	67	120
		2018	15	30	55	70	120
Immunology	4172	2016	10	30	60	80	128
		2017	10	30	60	79	123
		2018	15	30	60	77	120
Coagulation	4552	2016	10	30	40	60	120
		2017	10	30	43	60	120
		2018	10	30	45	60	120

B-043

The Use of Analytical Quality of a Test versus Its Perceived Clinical Quality as Performance Indicators: Development of an Automated Computer Program

S. Chakraborty, R. Bhattacharya, S. Rath. *Tata Medical Center, Kolkata, India*

Background: It is difficult to define and document the perceived clinical quality of a test result. In spite of the knowledge that most errors occur in the pre-analytic phase of the testing cycle, most laboratories limit their focus on quality assurance in the analytical phase only. Even though, commercially available programs are available to generate key performance indicators (KPI), in addition to a commercial burden they most often are not customizable. Many laboratories, in developing countries, thus shy away from using such programs. We have developed our own computer program in order to document the perceived quality of a test versus its analytical quality using measures of Six Sigma. The study was conducted in a tertiary cancer care hospital in India over a period of six months (August 2018 - January 2019) for serum sodium (Na), potassium (K), thyroid stimulating hormone (TSH), and total prostate specific antigen (PSA).

Methods: A standalone program for data entry and metric calculations was built using Python programing language. Defects and pivotal opportunities were documented in each phase of the testing process. The output was generated as Rolled Throughput Yield (RTY), Six Sigma Metric of the test and the perceived sigma metric of the test quality by the clinician. The RTY, Six Sigma performance of the test, and the perceived clinical Six Sigma characteristics were adopted as the KPIs.

Results: Barring serum Na (2.8 sigma), all other tests had 6 sigma performance. The RTY was highest for serum PSA (0.999 - 1.00), and least for serum TSH (0.994 -0.995), serum Na and K had values of (0.996 - 0.998) and (0.996 - 0.997) respectively. In spite of high analytical sigma and RTY for serum TSH, PSA, and K, their clinical sigma metric was varied from 4 - 4.8. Serum Na, on the other hand, had the lowest analytical sigma but the highest clinical sigma metric (6.00). The reworked utility of defective results was excluded from the perceived clinical utility of the test.

Conclusion: We have shown that the perceived clinical quality of a test may be different from that of its analytical quality. Serum Na has the lowest analytic sigma but the highest perceived test quality. The scenario is opposite with the other analytes. The developed computer program enables generation of the performance indicators by the technologists with ease. These KPIs have the capacity to identify areas of improvement such as staff training or physician education. In the resource-constrained settings of developing countries, this program has the potential for widespread adoption and use.

B-045

Design and Performance of ASAT and LDH Reporting Algorithms for Hemolyzed Specimens including Correction within Quality Specifications

S. Colak, O. Tasdemir, F. Opdam, D. van den Broek, H. van Rossum. *Netherlands Cancer Institute, Amsterdam, Netherlands*

Background: In vitro hemolysis is a major operational challenge for medical laboratories. A new experimental design was used to investigate under what conditions algorithms could be designed to report either quantitative or qualitative ASAT and LDH results outside the manufacturer's hemolysis specifications. Quantitative corrections were required to meet pre-specified quality specifications. **Methods:** Blood drawn from 25 patients was used to determine patient sample-specific effect of hemolysis on ASAT and LDH concentrations. ASAT, LDH and hemolysis index were determined using a Cobas 6000 analyzer. Correction factors were determined and the accuracy of the correction was investigated. Reporting algorithms were designed based on i) the manufacturer's cutoff for the hemolysis index, ii) corrections within the total allowable error specification and iii) qualitative reporting based on obtained results. The performance of the reporting algorithms was retrospectively determined by recalculating 6 months of ASAT and LDH results. **Results:** All generated hemolytic samples had ASAT/LDH results equal to or greater than the original result. Furthermore, no correction for ASAT/LDH results below the upper limit of normal was possible, while results equal to or greater than the upper limit of normal could, up to mild hemolysis, be corrected within the total error criterion. The reporting algorithms allowed reporting 88.5% and 85.9% of otherwise unreported ASAT and LDH results, respectively. **Conclusions:** An approach is presented that allows to generate evidence-based reporting algorithms for ASAT and LDH compatible with pre-specified quality specifications. The designed algorithms resulted in a significant reduction of otherwise unreported ASAT and LDH results.

B-046

Design and Implementation of Quality Control Plans that Integrate Moving Average and Internal Quality Control: Incorporating the Best of Both Worlds

H. van Rossum, D. van den Broek. *Netherlands Cancer Institute, Amsterdam, Netherlands*

Background: New moving average quality control (MA QC) optimization methods have been developed and are available for laboratories. Having these methods will require a strategy to integrate MA QC and routine internal QC. **Methods:** MA QC was considered only when the performance of internal QC was limited. A flowchart was applied to determine, per test, whether MA QC should be considered. Next, MA QC was examined using MA Generator, and optimized MA QC procedures and corresponding MA validation charts were obtained. When a relevant systematic error was detectable within an average daily run, the MA QC was added to the QC plan. For further implementation of MA QC for continuous QC, MA QC management software was configured based on earlier proposed requirements. Also, protocols for MA QC alarm work-up were designed to allow detection of temporary assay failure based on previously described experiences. **Results**: Based on the flowchart, 10 chemistry, 2 immunochemistry and 6 hematological tests were considered for MA QC. After obtaining optimal MA QC settings and the corresponding MA validation charts, the MA QC of albumin, bicarbonate, calcium, chloride, creatinine, glucose, magnesium, potassium, sodium, total protein, hematocrit, hemoglobin, MCH, MCHC, MCV and platelets were added to the QC plans. **Conclusions:** The presented method allows the design and implementation of QC plans integrating MA QC for continuous QC when internal QC has limited performance.

B-047

Proposed 2019 CLIA Proficiency Testing Limits: A Retrospective Review of Laboratory Performance using Current and Proposed Criteria of Acceptability

C. S. Norton-Wenzel, E. G. Baker, R. Rej. *Wadsworth Center, New York State Dept. Health, Albany, NY*

Background: Early this year proficiency testing regulations that proposed revised acceptable performance criteria under CLIA'88 were published [*Federal Register* V84(23), 4 February 2019]. While the frequency of proficiency tests and number of challenges per event remain unchanged, in general, the proposed acceptable limits are narrower than those currently in use. We examined the effects of implementing the proposed criteria on evaluation of laboratories.

Methods: Using data obtained from proficiency tests carried out by the New York State Department of Health over the period 2014-15 we compared laboratory performance using both the current criteria and the retrospective application of the new proposed criteria to the same data sets for several measurands.

Results: Representative laboratory performance using each set of criteria are shown in Table 1; the percentage of unacceptable results (miss rate) in columns 3 and 6 and the percentage of participants with unsatisfactory CLIA analyte scores (<80%) in columns 4 and 7. Data were from the January 2014 testing event. For cardiac troponins, γ-glutamyltransferase, and phosphorus there are no current acceptable criteria. The proposed acceptable range for amylase decreased by a factor of three (from ±30% to ±10%) and the number of unacceptable results increased from 1(0.06%) to 49(3.2%) of 1555 results.

Conclusion: For many measurands the acceptable performance criteria published in the proposed regulations are considerably tighter than those currently in use; e.g. from ±25% to ±15%. As anticipated there was an increase in the number of unacceptable results with the proposed criteria but the unacceptable rate remained below 2% in most cases, including the newly added measurands. In many cases the concentration of the measurand in the proficiency samples has an effect on acceptable rates and we are continuing this review to include a larger data set covering a wider concentration range.

Table 1. Proficiency Testing Performance Using Current and Proposed CLIA'88 Acceptable Criteria

Analyte	Current Limit	Unacceptable results % (Current)	Unsatisfactory participants % (Current)	Proposed Limit	Unacceptable results % (Proposed)	Unsatisfactory participants % (Proposed)
Glucose	±10% or ±6 mg/dL	0.6	0.5	± 8%	1.5	1.3
Creatinine	±15% or ± 0.3 mg/dL	0.6	0.8	±10% or ±0.2 mg/dL	1.1	1.3
Triglycerides	±25%	0.4	0.6	±15%	1.6	1.6
Magnesium	±25%	0.2	0.3	±15%	1.2	2.1
Aspartate aminotransferase	±20%	0.6	0.6	±15%	0.9	1.1
α-Amylase	±30%	0.1	0.0	±10%	3.2	3.2
Alkaline Phosphatase	±30%	0.4	0.0	±20%	2.7	2.0
Phenobarbital	±20%	1.0	0.1	±15%	3.0	2,6
Phenytoin	±25%	0.1	0.0	±15% or ±2 μg/dL	2.1	1.9
Vancomycin	±25%	0.7	0.1	±15% or ±2 μg/dL	2.2	2.7
Troponin-I	-	-	-	±30% or ±0.9 ng/mL	0.1	0.0
Troponin-T	-	-	-	±30% or ±0.2 ng/mL	0.0	0.0
γ-Glutamyltransferase	-	-	-	±15% or ±5 U/L	1.0	1.4
Phosphorus	-	-	-	±10% or ±0.3 mg/dL	1.5	2.1

B-048

A Medical Consulting Center as a Strategy for Customer Relationship Management and Adding Value to the Physician and the Patient

P. Araujo, A. Salvador, R. Fontes, Y. Shrank, A. Perozo, D. Rodrigues, L. Santos, A. Bispo, J. Souza, L. Hasselmann, E. Carneiro da Silva, R. Sousa Rocco, G. Campana, M. di Calafiori Freire. *DASA, Brazil, Rio de Janeiro, Brazil*

Background: A structured relationship between the clinical laboratory and prescribing physicians is critical to the organization success, since it plays a role on the perception of value and reliability of laboratory results. Following this premise, a private laboratory located in Rio de Janeiro, Brazil, focused in this process establishing a Medical Consulting Center (MCC) for clients, composed of doctors and biomedicals, which is responsible for the reception of prescribing physicians' demands, as well as for the prompt resolution of internal occurrences and notifications that are of medical interest.**Objectives:** Our aim was to evaluate the outcomes of the activities performed by the MCC, designed to provide a high quality customer relationship management (CRM), adding value to laboratory medicine.**Methods:** In order to measure the performance of this department, 4 indicators were established by the leadership and the team and obtained on a monthly basis. To evaluate the fidelity of the end customer, net promoter score (NPS) surveys and satisfaction assessments were conducted with patients and prescribing physicians, separately. We studied the performance and outcomes of this process from January to December 2018.**Results:** During the year of 2018, MCC received a monthly average of 5599(± 705) telephone calls, being 70% from physicians and 30% from patients. The dropout rate goal was less than 3%, and result was 0.46%. Other indicators performed as follows (monthly average): notification of the need for a new sample for medical within 48 hours was 100% (225/225 - goal over 98%); medical requests or complaints replied to the requesting physician within 12 working hours was 100% (66/66, goal over 95%), notification of altered test results of short term patient health impact within 5 business days was 89.9%

(293/327, goal over 80%). Regarding NPS, different goals are set for doctor's and patient's surveys, and for each of the 3 different private laboratory brands, since they are designed to different population niches and service levels at the drawing facilities. Thus, for the doctor NPS the attainments and goals for the 3 lab brands were: standard 72.8/72.0; executive/standard 79.1/74.5; executive 81.2/80.0, and the patient NPS performance were, respectively, 69.7/67.5; 80.3/78.6; 76.3/76.0. The war map of the satisfaction survey showed the relationship between the attribute of value and the given NPS by the responder: "test results making sense with clinical findings" (33% of choices/NPS 80), "medical staff quality" (6%/85), "quality of the MCC service" (3%/78) and "promptness of access to MCC" (4%/79). **Discussion:** We showed that the attributes related to MCC processes contribute to higher NPS results. The high level performance of the MCC represents opportunities to win over and retain clients, especially the doctor, since prescribers need to rely on the laboratory results used in their medical practice, and medical discussion with pairs in the lab is an important part of it. In addition, physicians may influence the patients' choice of laboratory. Thus, an easy and efficient communication channel between the MCC, composed of experienced medical specialists, and the doctors, adds value to the final medical product.

B-049

Automated Clinical Chemistry Platform Performance Across Two Academic Medical Centers

M. L. Brown[1], J. M. Colby[2], M. Feldhammer[1], J. H. Nichols[2]. *[1]University of Florida, Jacksonville, FL, [2]Vanderbilt University Medical Center, Nashville, TN*

Objective: We sought to define the performance of automated chemistry platforms at two large academic medical centers by calculating and comparing sigma metrics for 28 analytes.

Methods: Performance characteristics of chemistry assays on two Roche Cobas analyzers (University of Florida Health Jacksonville) and four Abbott Architect analyzers (Vanderbilt University Medical Center) were estimated using 12 months of Bio-Rad quality control (QC) data at two concentrations. Method imprecision was calculated as the cumulative QC coefficient of variation (CV) across analyzers at each QC concentration and percent bias was calculated by comparison of analyzer QC mean to peer group means. Sigma values were calculated for each method as [(TEa - Bias%)/CV%] using allowable total error (TEa) from two sources: the CLIA evaluation limits and desirable biological variation (Ricos C et al.). Average sigma values were generated for each site and graded as optimal >6 sigma; good 5-6 sigma; marginal 3-5 sigma; or poor <3 sigma. Analysis of NIST SRM1950 standards for a subset of analytes allowed an estimation of absolute bias.

Results: Sigma metrics were highly comparable across both study sites. Considering CLIA TEa, just over half (UF 57%; VUMC 54%) of the 28 analytes met the six-sigma standard of performance. The highest performing groups to meet or exceed the six-sigma standard were lipids (cholesterol, LDL, HDL and triglycerides) and enzymes (ALP, ALT, AST, CK, GGT, LDH and lipase). Electrolytes (Na, K, Cl, Mg) and metabolites (total bilirubin, BUN, CO2) failed to meet six-sigma. Notably, there were dramatic differences in sigma values calculated using CLIA and Ricos TEa criteria. Almost 40% of the analytes had at least one QC that performed poorly using Ricos criteria. Only 4 of the 28 assays (CK, GGT, Lipase and triglycerides) demonstrated optimal performance at both study sites using Ricos and CLIA criteria. Overall, eleven analytes at each institution exhibited unacceptable performance using Ricos criteria as opposed to only 2 analytes with the CLIA TEa limits. Analysis of NIST SRM1950 at both study sites gave

comparable sigmas for all analytes except total bilirubin, cholesterol, Mg and total protein.

Conclusion: Neither Abbott nor Roche analyzers meet six-sigma quality standards for all analytes tested. Overall assay performance across these two platforms at two major academic medical centers was almost identical. CLIA TEa and RICOS TEa criteria are significantly different, with wider limits of acceptability for CLIA. Variations between individual analyzers and manufacturers and limitations in automation would make tailored QC rules based on sigma metrics difficult to implement in a high-volume laboratory.

B-050

Self-Perception of Competencies and Need of Training in Evidence Based Medicine among Medical Laboratory Professionals Worldwide

K. Rodriguez-Capote[1], N. Gimenez[2], C. Florkowski[3], A. Don-Wauchope[4], J. Wils[5], A. Zemlin[6], C. Evidence-Based-laboratory-Medicine-IFCC[7]. *[1]University of British Columbia, New Westminster, BC, Canada, [2]Universitat de Barcelona, Barcelona, Spain, [3]Canterbury Health Laboratories, Christchurch, New Zealand, [4]LifeLabs, Toronto, ON, Canada, [5]University Hospital of Rouen, Rouen, France, [6]University of Stellenbosch and National Health, Cape Town, South Africa, [7]International Federation of Clinical Chemistry, Milano, Italy*

Background: To investigate the perceptions of medical laboratory professionals worldwide regarding their competences and needs in Evidence Based Medicine (EBM). **Methods:** A self-completed questionnaire was designed by the Evidence -Based Laboratory Medicine Committee (EBLM) of the Spanish Society of Laboratory Medicine (SEQC-ML) and adapted to English by the EBLM Committee of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC). The questionnaire consisted of six sections (A. Sociodemographic Data; B. Training in EBM; C. Access to EBM Material; D. Perceptions about Self-Competency; E. Preference for Training and F. Comments and Suggestions) and 36 variables. The variables were scored on a scale of 1 to 10. The survey was administered via SurveyMonkey® and mailed in April 2017 to all Presidents and Representatives of IFCC National Societies. Data analysis was performed using IBM SPSS. The study was performed in accordance with the Ethics Research Committee.

Results: 355 IFCC members responded to the survey. The mean age of respondents was 48 years, the mean professional experience was 21 years and 53% were female. 54% of respondents were Europeans, 25% Americans, 20% Asians, 2% Africans and 1% Australians, 80% reported to have some basic training, 16% advanced training and 3% did not respond. α-Cronbach's coefficient=0.860. Medical laboratory professionals indicated that they had a low level of satisfaction with their current knowledge of EBM (average 4.62 points). There is a great interest in learning how to access Evidence Based Clinical Practice Guidelines (average 8.14 points) and in receiving further training on EBM (average 7.77 points). Factors that showed differences in responses were gender, formal training in EBM, years of experience and age. Men were more satisfied about their EBM knowledge than women (5.21 points vs 4.45 points; p=0.004) and women were more interested in improving their knowledge (8.26 points vs 7.75 points; p=0.034)

Conclusion: Medical laboratory professionals were very interested in EBM, especially in accessing Clinical Practice Guidelines and other EBLM materials. They also considered EBM is necessary in their daily practice. The findings of this survey will help to plan future training on EBM for laboratory professionals.

B-051

Prevalence of Low Serum Sodium Levels at Hospital Admission

M. F. M. C. Pinheiro, T. S. P. Souza, V. V. Tessari, L. C. Vieira, G. H. C. C. Escanilha, R. F. M. Souza, M. C. Freire, G. A. Campana. *DASA, Rio de Janeiro, Brazil*

Background: Hyponatremia is the most frequent electrolyte disorder among hospitalized patients. It is associated with poor outcomes such as increased mortality, prolonged length of hospital stays, and increased healthcare costs. While it is not clear whether there is a direct causal relationship of hyponatremia with adverse outcomes or it is simply a marker of disease severity, it is well-recognized that improper management of a hyponatremic patient may result in severe neurologic damage or death. **Objective**: To evaluate the prevalence of hyponatremia at hospital admission. **Methods:** We analyzed all blood samples requesting serum sodium levels from patients admitted to a 120 bed high complexity tertiary hospital in Rio de Janeiro from May 2015 to December 2018. For this study, we examined only the first serum sodium (Na) test at hospital admission. Anonymized data on laboratory tests and demographic variables were available from a database of the Laboratory Information System. To determine the severity of hyponatremia (sodium reference value: 135 to 145 mEq/L), we separated patients according serum sodium concentration: severe, <120 mEq/L; moderate, 120 to 129 mEq/L and mild, 130 to 134 mEq/L. We categorized age into four different groups: 18 to 39, 40 to 59, 60 to 79 and >= 80 years old. **Results**: We studied 15,961 patients, 52.1% female, mean age 56.8 (± 21.3) yrs. Of these, 27% were aged 18 to 39 yrs, 26.4% from 40 to 59 yrs, 27.8% from 60 to 79 yrs and 18.8% were older than 80 yrs. Serum Na (mean 137.7 ± 3.7) ranged between 108 and 178 mEq/L. Hyponatremia, normal and hypernatremia were observed in 14.9 %, 84.5 %, 0.6 % in females versus 12.2 %. 87.0 % and 0.8 % in males, respectively. The fre-

quency of hyponatremia was higher in females (p<0.05). Hyponatremia on admission was mild in the majority of patients (79.3%), moderate in 18.9 % and severe in 1.8 %. Compared the mean of patients with normonatremia, those with hyponatremia were significantly older (mean age 54 vs 71 years, *P* <0.001). In the group of severe hyponatremia, 51.2% were older than 80 yrs. **Conclusions:** In accordance to previous studies, hyponatremia is a frequent electrolyte disorder at hospital admission, especially in older people. Our results underscore the need to be alert to the sodium levels, as hyponatremia is seen frequently and can be associated with poor outcomes.

B-052

Value of Test Volume Benchmarking for Utilization Management and Laboratory Stewardship

R. Schifman[1], J. Y. Wang-Rodriguez[2], D. R. Luevano[1]. [1]*Southern AZ VA Healthcare System, Tucson, AZ,* [2]*VA San Diego Healthcare System, San Diego, CA*

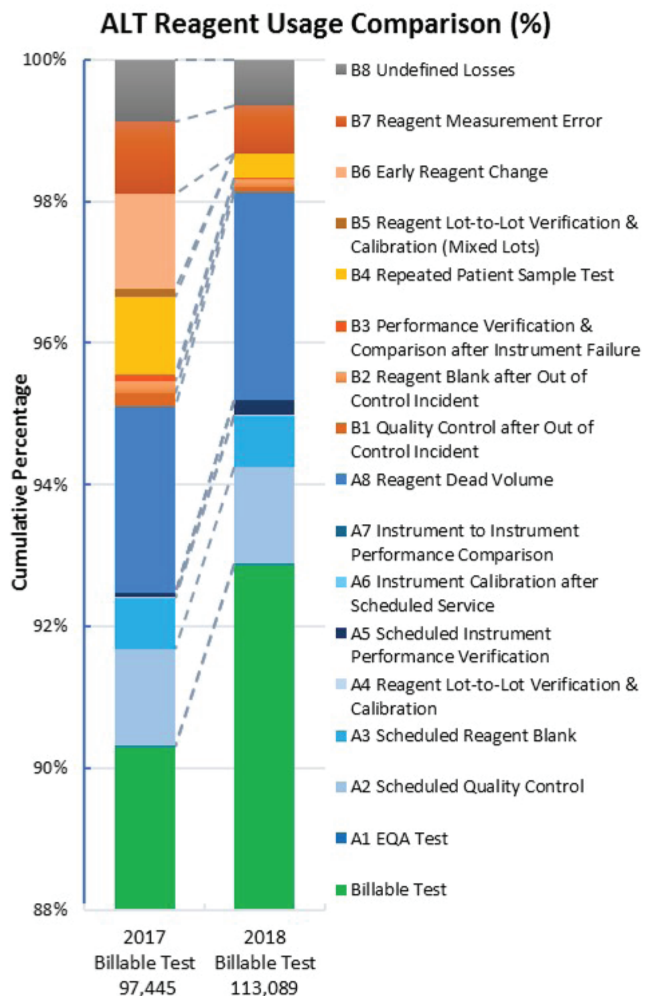
Background: It is well established that test ordering practices vary widely between healthcare providers even when adjusted for similar patient populations and conditions due, in part, from operational practices and policies. This may involve test menu configuration, ordering protocols or restriction policies. The objective of this study was to evaluate variance in annual volume for specific tests among facilities as an indicator of potential utilization problems and opportunity for practice improvements. **Methods:** Annual serum sodium test volume was compared to total unique patients and used as denominator to adjust for variation in total test volume between facilities Sodium-adjusted annual volumes for multiple analytes were compared between 8 facilities within a Veterans Affairs healthcare network. Each facility was provided benchmarking results and encouraged to evaluate adjusted test volume outliers for potential utilization problems. **Results:** Total unique patients served among facilities ranged from 22,000 to 79,000 and annual reported serum sodium results from 42,400 to 179,300. There was good correlation between serum sodium volume and unique patients (r2=0.90, y=0.406x + 3595) among the 8 facilities evaluated. Test volume outliers included 1) Alpha-1 antitrypsin phenotype: 2.35/1,000 sodium vs. <0.01/1,000 for others; this was due to phenotype being used for screening and was replaced with quantitative testing by immunoassay. 2) Serum aluminum: 2.4/1,000 vs <0.6/1,000 sodiums for others - this was due to testing frequency (quarterly) which was reduced to 1-2 times/year in dialysis patients per guideline recommendation. 3) Serum LDH: 11.5/100 vs 0.6-4.5/100 sodiums - due to configuration of “liver panel, ” LDH removed. 4) BCR/ABL1: 2.6/1,000 vs. 0.6-1.3/1,000 sodiums - due to separate p190 and p210 mutations being ordered together as panel. Practice changed to primarily single mutation ordering. Finally, CK-MB showed widest variation among facilities; 52-79/1,000 (n=4) vs. <1.0/1,000 sodiums for other 4 facilities. This information was successfully used by one facility to support primary use of troponin only as diagnostic evaluation of chest pain. **Conclusion:** Use of annual test volume comparisons when adjusted against a high-volume test (serum sodium) provided actionable information for identifying gaps in test ordering practices within our healthcare network. Additional work is needed to determine if this method is similarly applicable among other types of healthcare facilities and practices. Stratification by patient location (inpatient vs outpatient), and complexity of care among facilities deserve further investigation. In conclusion, test volume benchmarking in our VA healthcare network proved to be an Important adjunct for laboratory stewardship that lead to utilization improvements.

B-053

Management of Reagents in Lean Laboratory

H. Sui[1], Y. Hong[2], N. Xiu[1], W. Chen[1]. [1]*Dongguan Kanghua Hospital, Dongguan, China,* [2]*London Business School, London, United Kingdom*

Background: Laboratories are under pressure to deliver timely, high-quality and cost-efficient tests with stretching resources. As reagents cost is a main contributor of laboratories operating budget, this study adopted Lean Six Sigma methodology to improve value-added reagent utilization by determining root causes of unnecessary reagent consumptions (URCs), monitoring and minimizing reagent wastes. **Methods:** Reagents of 3 assays, ALT (Siemens), D-Dimer (Sysmex) and PCT (ROCHE), were selected. Value Stream Mapping (VSM) and Root Cause Analysis are used to expose, categorize the URCs and identify root causes. Reagent usages collected and compared from 07.2017 to 12.2017 and from 07.2018 to 12.2018. Design for Six Sigma framework was applied to re-design the process and develop the Reagent Monitoring Module (RMM) based on Siemens Centralink Data Management System. **Results:** URCs have been categorized into two types: A) Usages for scheduled QC, instrument calibration and dead volume; B) Usages for repeated patient tests and incident related tests (QC after out-of-control event). VSM revealed study focus to be Type-B waste which would contribute more reagent saving. Root causes identified were inadequate reagent tracking at instrument level and insufficient instrument warning prompting system. The RMM was employed to track real-time reagent usage and to centralize warning information for all instruments. Process and system improvements resulted in an increased average value-added utilization of 3 reagents from 88.7% to 92.3%, with reductions of 0.1% and 3.5% of Type-A and Type-B wastes respectively. Real-time reagent management system raised utilization by 1.6%, 0.6% was attributed to advanced instrument maintenance system, while 0.8% was due to reduced patient sample retests. The cause of undefined losses and the 0.5% utilization increase are under investigation. The graph demonstrates utilization comparison of ALT reagent between 2017 and 2018 with detailed breakdown of URCs. **Conclusion:** This study has improved value-added reagent utilization, reduced cost per test, while maintaining timely, high-quality service.



B-054

Improved Laboratory Compliance to Quality Standards through On-Site Trainings in Cambodia

S. Ong[1], S. Song[1], N. Ndefru[2], P. Sadate-Ngatchou[2], S. Sau[3], L. Perrone[2]. [1]*International Training and Education Center for Health (I-TECH), Phnom Penh, Cambodia,* [2]*University of Washington, Seattle, WA,* [3]*Ministry of Health, Phnom Penh, Cambodia*

Background: International Training and Education Center for Health (I-TECH), University of Washington, has contributed in strengthening laboratory quality in Cambodia since 2014. Using Laboratory Quality Stepwise Implementation tool, I-TECH mentored 12 national laboratories in accordance with the requirements of ISO 15189. In 2017 at the onset of the 2nd phase of our project (after a year lapse in implementation laboratory quality strengthening), we assessed all our 12 target laboratories.

These audits revealed gaps in non-compliance to laboratory quality management system (QMS) including in equipment maintenance. Lack of staff engagement to improve QMS was noticeable. Although standard operating procedures (SOPs) were written, laboratory staff had not honed SOP implementation at the bench.

Methods: I-TECH mentored 12 laboratories with the aim to improve staff knowledge in QMS by identifying areas that showed the least staff engagement during the audits. We organized and conducted on-site daylong trainings at three workshops. The 12 laboratories were divided into 3 regional groups. Each workshop trained 8 trainees including Laboratory Directors and Quality Managers. Total of 40 laboratorians attended the three workshops. Trainings consisted of walking through the laboratory to check for non-conformities and a hands-on practicum on correct pipetting skills, with lectures on reagent grade water quality and distiller equipment maintenance. Training participation was documented, and attendees evaluated workshop trainings.

Results: Non-conformities documented at all 12 mentored-laboratories included the following: all 12 laboratories did not perform pipettes accuracy check; had inadequately maintained distillers; distilled water container label was not standardized according to the globally harmonized system. One month after the three on-site training workshops, an assessment showed that 25% (3/12) laboratories have completed accuracy checks on all their pipettes. Two laboratories have purchased the opaque containers for storing the distilled water. One laboratory had purchased a new distiller and negotiated a maintenance program. Training evaluation indicated that all participants agreed on the relevance of the training content to their work, they all learned new skills, and they would recommend this training to other colleagues.

Conclusion: The three on-site training workshop methodology showed positive learning and improvement of the laboratory processes.

B-055

MDIC Clinical Dx IVD Clinical Evidence Framework

C. Hiller. *Medical Device Innovation Consortium, Arlington, VA*

Background: The Medical Device Innovation Consortium (MDIC) is the first-ever public-private partnership created with the sole objective of advancing the regulatory science around the development and assessment of medical devices (which include in vitro diagnostics [IVDs]).

Market entry for an IVD test requires evidence of analytical and clinical validity: how well the test predicts the presence, absence or risk of a specific condition in order to get the analyte cleared or approved by FDA. Payer coverage and provider adoption also require evidence of clinical utility: whether the information that a test provides is useful to improve the management and outcome of a condition.

Methods:

A working group consisting of FDA, IVD industry, and payer representatives collectively worked to develop a framework on developing evidence. For an In Vitro Diagnostic (IVD), analytical and clinical validity are required for FDA clearance/approval, and clinical utility is required for payer coverage and provider adoption. Many sponsors separate data collection for these purposes, often waiting until after regulatory clearance/approval to collect data for clinical utility, which delays market entry or can limit coverage. Furthermore, that stepwise approach means that narrow intended uses may facilitate approval/clearance through the FDA, but make the demonstration of clinical utility more difficult, requiring the duplication of some data collection and or subsequent changes in the intended use and increase FDA regulatory burden. Defining the pathways for generating sufficient evidence of analytical and clinical validity in parallel with clinical utility can potentially increase the efficiency and reduce costs of IVD development.

The Framework is organized into five sections, outlined below:

Section One: Introduction - introduces the framework, definitions of analytical validity, clinical validity, and basic concepts of FDA clearance and approval of medical devices, including IVDs.

Section Two: Analytical Validity - provides a list of studies frequently used to demonstrate analytical validity. Useful terms, test considerations and requirements, and related references are provided.

Section Three: Clinical Validity - discusses assay types and measures of clinical validation, as well as design considerations for clinical validation based on the intended use of the IVD.

Section Four: Clinical Utility - explores the general strategy for developing evidence of clinical utility for payers, presents a clinical utility “self-assessment” framework that IVD developers can use for planning, and details additional clinical utility considerations by IVD test type.

Section Five: References - lists regulatory documents not provided in-line with the text and other references cited in this Framework.

Results:

The MDIC IVD Clinical Evidence Framework provides a high-level overview that IVD manufacturers can use to make decisions on how to develop credible evidence of analytical and clinical validity as well as clinical utility.

Conclusion:

This Framework is intended to help IVD manufacturers make decisions on how to develop credible evidence of analytical and clinical validity, as well as clinical utility.

This Framework should be considered an initial thought piece and not a prescriptive, “how-to” guide. Reading and following this document neither guarantees FDA approval/clearance nor payment from insurance companies.

B-056

Lean Daily Management Tool to Assess Procalcitonin Compliance in an Antimicrobial Stewardship Sepsis Program

B. Cook, K. Macri, S. Rodgers, L. Stezar, H. Zari, J. Zajechowski, J. Copeland. *Henry Ford Hospital, Detroit, MI*

Background: Lean daily management was used to measure provider adherence to procalcitonin (PCT) ordering guidelines published in a Tier 1 system-wide policy for an antimicrobial stewardship sepsis program. Sepsis is a deadly condition, with >1.5 million cases and 250,000 deaths each year in the US. Antibiotic therapy is effective in saving lives and reducing morbidity. Due to high cost and side effects, removing patients from antibiotics ASAP is important. PCT has been demonstrated to effectively identify those patients who can stop receiving antibiotics.

Methods: Henry Ford Hospital is a tertiary care center, part of a health system with 4 other hospitals in Southeast Michigan. Emergency centers across the health system assess >1000 cases monthly for potential sepsis. The departments of Infectious Disease, Pharmacy, and Pathology incorporated PCT testing in an antibiotic cessation program in July 2017. This followed a Tier 1 Policy that provides algorithm-guided approach for managing the duration of antimicrobial therapy for patients with lower respiratory tract infections. PCT measurements are to be ordered every 2 to 3 days, but no more frequently than once daily. While PCT is an important triage and alert tool, the potential for waste, i.e., non-informative testing, is significant. The laboratory was receiving what appeared to be an increasing number of duplicate (<2-3 days) PCT orders from providers who were ordering more than once in a 24-hour period. From this early assumption of possible system waste, we monitored PCT orders frequency using a visual daily management metric board to track order frequency, daily, and monthly trending through Pareto charting for order location and root cause and to track corrective action intervention.

Results: Preliminary surveillance of PCT ordering volumes determined orders for PCT increased significantly across the health system, from 62 per year as a send-out test to 6748 per year once testing was offered in-house. Approximately 35% of tests were ordered at our main hospital and the remaining 65% at the other Henry Ford locations. Duplicate orders accounted for 12% of annual orders, potentially representing significant waste and low-value clinical information. Based on this preliminary assessment, we initiated an improvement project to track duplicate PCT orders and identify ordering site, service, and provider. Data was collected for a 6 month period, with periodic investigating for trends or site-specific anomalies. On an annualized basis, providers ordered ~19,600 PCT tests producing 720 (3.7%) duplicate tests, a lower fraction than observed in the preliminary assessment. When duplicate orders were examined by ordering site, the distribution matched the total PCT orders by site, with no one site exhibiting trends of duplicative orders. Analyzing duplicate orders by provider showed no significant outliers; single-instances accounted for the majority (78%) of duplicate PCT orders. Those providers with 2 and 3 duplicate orders accounted for 14% and 7%, respectively.

Conclusions: Surveillance using daily management metrics ensured our system policy was effective in limiting wasteful, duplicate PCT orders thus conserving laboratory productivity, provider review time, and minimizing unnecessary patient discomfort.

B-057

Healthcare Kaizen: A Process Improvement Case Study in a Medical Laboratory Company

J. M. G. Faria, F. B. Pena, A. C. Rocha, D. Waltrick, G. Grejianin, C. O. W. Sabino, L. Rodrigues, E. A. Perobelli, I. C. A. Souza, D. C. C. Akiyama, K. C. Magalhaes, A. F. J. Feo, P. S. Osorio. *DASA- DIAGNOSTICOS DA AMERICA, BARUERI, Brazil*

Background: Motivated by an organizational strategic guideline (Objective Key Result - OKR) of having the most efficient medical laboratory operation in the World, continuous improvement process and practices innovation were adopted through kaizen methodology to support employees to address complex operational issues in order to reduce cost, improve quality and maintain process reliability. **Objective**: Reduce unnecessary tests repetition criteria of clinical exams.

Methods: In order to ensure quality and clinical premises of the exam results, besides clinical analysis production department, were invited to join the kaizen team the following departments: integrated quality, medical consultant staff and laboratory personnel along with information technology representants. The project scope used a *Pareto Principle* for the clinical analysis production areas and exams, which have higher levels of repeat testing. Approximately 400 exams compounded by biochemical, hormonal and immunological automated clinical analysis with a pre-established repetition rules in the laboratory information system (LIS) were analyzed. With the project scope, were defined as correction criteria (criteria for a new repeating rule), test linearity, statistical evaluations of patients result, results reproducibility, and manufacturer information. All these criteria were reviewed and re-analyzed by the company medical area to guarantee the correct clinical significance of the released results.

Results: From 400 exams, 50 fulfill the criteria, about 13% of the total analyses studied. This analysis showed significant reduction of tests repetition, around 30%, with direct impact the reduction of the test unit cost, around 19% of the annual total cost defined by the project scope. Also, it demonstrated the need to consolidate process standardization for new LIS repeat rules, creating a new scope for monitoring the existing rules, formatting approvals flow for changes and follow-ups, and creating a book of repetition criteria to standardize the company's laboratory information system (LIS) that will lead all changes to repetition rules, platforms and methodologies for all tests with LIS interface. This book will be managed by the Integrated Quality sector of the company to mitigate regionally. Moreover, an agile mindset was applied in order to have a high level of delivery and business benefit in a short sustainable time. For example, the use of the Minimum Viable Product (MVP) concept to develop the standard process for new LIS repeat rules and for the book of repletion criteria.

Conclusion: The Kaizen methodology, chosen to develop and implement the project in the company, proved to be efficient and agile, returning short-term immediate actions for continuous improvement of quality and increase of revenue, with constant updating of the actions taken in the project.

B-058

Post-Analytical Proficiency Testing - Experience with Interpretative Clinical Cases for Porphyria Investigation

G. J. Davies, M. A. Thomas, S. Jones, L. J. Davison, T. Rees. *Cardiff and Vale UHB, Cardiff, United Kingdom*

Introduction

Proficiency testing is an essential part of assuring the quality of laboratory diagnostic services, and should where possible include assessment of both pre and post analytical phases of the diagnostic cycle.

Method

As part of the Weqas Porphyrin programme, laboratories were issued with 3 urine samples containing Porphobilinogen (PBG) along with relevant clinical information for each sample. Laboratories were asked to analyse the sample and provide interpretive comments suggesting further investigation if appropriate. Participant responses were assessed for both analytical and post-analytical performance. The correct interpretation was provided by an expert advisor and made available to all participants along with a summary of the responses. Key phrases used by the expert are used to evaluate the responses.

Results & Discussion

Case 1 was a known VP patient with abdominal pain and vomiting, PBG/Creatinine ratio overall mean 23.4 µmol/mmol. According to the expert opinion this confirms a current acute attack. 11 of 14 users stated that this was consistent with a current acute attack, with further details of specialist advice centres provided by 5 users. Case 2 was a known AIP patient with abdominal pain, PBG/Creatinine ratio overall mean 5.5 µmol/mmol. This PBG elevation is not typical of an acute attack, and more consistent with the smaller elevations seen in remission / latent phase of the disease. 10 of 14 users stated that this was elevations seen in between acute attacks, 4 stated results do not exclude or confirm an acute attack, and further details of specialist advice centres were provided by 5 users. Case 3 was a random urine sample from the mother of an AIP patient, PBG/Creatinine ratio overall mean 0.8 µmol/mmol. The normal PBG does not exclude latent porphyria in a family member of an affected patient and further investigation is required. 5 of 14 users stated that the results exclude a current acute attack, all 14 stated that latent porphyria could not be excluded, and all 14 suggested further studies and discussion with the National Acute Porphyria Service.

Conclusion

The programme has highlighted the importance of assessing the post-analytical phase and demonstrates the variability of expertise in reporting Porphyrin results within the UK.

B-059

Performance Evaluation of Anemia Assays on The Atellica IM Analyzer in Brazil

F. Brito, W. Pedrosa. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Two billion people - over 30% of the world's population - are anemic, many due to iron deficiency. In resource-poor areas, this is frequently exacerbated by infectious diseases, placing a significant health and economic burden on both patients and healthcare institutions. Since anemia is usually found during routine blood tests, laboratory diagnostic testing plays an integral role in addressing the various clinical needs for anemia management. Studies at our institution were performed to assess the analytical performance of Vitamin B12, Folate and Ferritin for the Atellica® IM 1600 Analyzer with respect to method comparison with Beckman Coulter DxI® System. **Methods:** Precision verification was performed according to EP15-A3, method comparison by EP09-A3. Method comparison studies were performed on 87 to 94 serum samples whose concentrations covered each assay range, from low to high. The results were analyzed using statistical tools and specifications of analytical quality. **Results:** Method comparison studies are summarized in Table below. The Vitamin B12 assay differences were most accentuated at lower vitamin B12 concentrations, being constant ranging from 0 to 32.8% from 500.0 pg/mL. For folate assay the differences were more accentuated up to 15.0 ng/mL of folate, from there being constant between -20.0 to + 16.2%. For ferritin assay the differences were more accentuated up to 200.0 ng/mL ferritin, from there being constant between +24.1 and +40.0%. **Conclusions:** All immunoassays tested on the Atellica IM 1600 Analyzer demonstrated good precision and correlation to the current DxI® System assay. The precision results were consistent with manufacturer's claims. **Siemens Healthineers supported the study by providing systems and reagents.*

Assay	Units	System Analyzer	Median	Method Comparison		
				Distribution of Differences	Linear Regression	Correlation Coefficient*
Vitamin B12	pg/mL	DxI®	324.9	-5.0% to +98.8% (+29.6%)	Y = (-101.867) + 0.993 X	0.962
		Atellica®	411.0			
Folate	ng/mL	DxI®	9.75	-29.6% to +126.8% (+9,0%)	Y = (-0.280) + 1.056 X	0.780
		Atellica®	11.57			
Ferritin	ng/mL	DxI®	63.8	-5.7% to +47.2% (+24,2%)	Y = (-1.546) + 1.21 X	0.998
		Atellica®	79.3			

*Spearman Coefficient

B-060

Performance Evaluation of Eletrolytes and Renal Function Biomarkers Assays on The Atellica CH 930 Analyzer

D. Waltrick, L. B. Avallone, L. Rodrigues, C. O. W. Sabino, C. M. M. Dias, E. A. Perobelli, G. A. Campana. *DASA, São Paulo, Brazil*

Background: The prevalence of kidney diseases continues to grow worldwide, mainly due to the population aging and the incidence of diabetes. Important for the diagnosis, prognosis, and monitoring of diseases affecting the kidneys, the assessment of renal function can be performed by measuring plasma concentrations of residual substances, such as creatinine, urea and electrolytes, and also the glomerular filtration rate (eTFG) that uses creatinine as a calculation parameter. Thus, the primary objective of this study was to demonstrate the analytical performance of the cited assays of renal function and electrolyte evaluation for use in the Atellica CH930 Clinical Chemistry Analyzer, through the routine results of a large laboratory in Brazil. Studies included precision evaluation, linearity, and method comparison. **Methods:** The Atellica CH Uric Acid, Chloride, Creatinine, Potassium, Sodium and Urea assays use the same reagents and calibrators from the respective assays in ADVIA Chemistry. The precision study was performed according to EP15-A3, the method comparison according to EP09-A3, and the linearity study according to EP06-A. For the precision study, two concentration ranges were used; each level of QC materials was tested in one run per day with five replicates per run for five days, resulting in a total of 25 replicates per sample for each run. Method comparison studies were performed using at least 40 serum samples that covered the linearity of the method. The number of linearity material levels ranged up to seven, depending on the assay. For each assay, they were tested in triplicate per sample concentration. **Results:** The precision results agreed with the manufacturer's claims. Repeatability CVs ranged from 0.546% to 2.965% and intra-laboratory CVs ranged from 0.575% to 3.181% among the kidney biomarkers tested from the Atellica CH analyzer. For the electrolytes, the repeatability CVs were 0.001% to 0.328% and the Run-to-Run CVs were 0.208% to 0.669%. The Passing & Bablock method comparison results ranged from 0.9543 to 0.9999. The dispersion of the results analyzed by the graph of the relative differences is within the total error allowed for all assays. Linearity results were obtained for Uric Acid, Creatinine and Urea. The assays tested on Atellica CH Analyzer and ADVIA Chemistry demonstrated excellent agreement. **Conclusion**: All assays tested on the Atellica CH 930 Analyzer demonstrated equivalence between the results of ADVIA Chemistry assays. The imprecision results are consistent with the manufacturer's claims and with the data available in the literature from the biological variation studies. Any creatinine values variation will be important for calculating eTFG to reclassify the stage of Renal Disease. In this way, reliable and accurate assays are required. The study demonstrated that there is no clinical impact on the trials tested in a possible migration for the Atellica CH 930 analyser. **Siemens Healthineers supported the studies by providing systems, and reagents.*

B-061

Daily Labs Quality Improvement: Pilot Program for Decreasing Unnecessary Lab Orders

E. Bachert, B. Breazile, T. Emmerich, A. Kelly, C. Castellanos, R. Chadha, A. Hall, P. Talari, A. Woodworth. *University of Kentucky, Lexington, KY*

Background: The Choosing Wisely Campaign from the American Board of Internal Medicine recommends against routine daily laboratory testing on hospitalized patients. Repetitive daily labs contribute to anemia, poor patient sleep, and increased infection risks. Furthermore, false-positive laboratory test results lead to unnecessary follow-up testing. Following these guidelines, our institution undertook a six month quality initiative aimed at decreasing daily laboratory test orders with no measurable patient harm.

Objectives: To implement a quality initiative to reduce routine daily laboratory orders without causing adverse patient outcomes.

Methods: Baseline values for laboratory test orders from inpatient internal medicine services were collected over a seven month period (February 1 to August 31, 2018) for five panels: comprehensive metabolic panel (CMP), basic metabolic panel (BMP), renal function panel (RFP), complete blood count (CBC), and complete blood count with differential (CBCPD). A multidisciplinary team from Hospital Medicine, Pathology, and the Data Analytics Core implemented a series of interventions with initial data review from November 1, 2018 to January 15, 2019. Interventions included: (1) initial presentations to the faculty and residents, (2) weekly and monthly emails sent to each inpatient service team comparing their weekly laboratory test volumes to the baseline average and to the other service teams, (3) a link to an online Tableau dashboard with filtering capability for laboratory test orders by service and provider, and (4) monthly project updates for house staff with announcement of the teaching team that decreased their lab test orders the most over the prior month. The project's goal was a decrease in laboratory test orders for the five panels above by 15%. Balancing measures from the pre- versus post-intervention periods included 30-day readmission rate, mortality rate, intensive care unit (ICU) transfer rates, and average hospital length of stay. Average Elixhauser Comorbidity Index, and case mix index were used to ensure that the pre- and post-intervention populations had similar degrees of illness.

Results: There were 6,651 unique inpatient visits for the pre-intervention period with an average of 0.665 BMP/CMP/RFP results per patient day and 0.560 CBC/CBCPD results per patient day. For the post-intervention period (November 1, 2018 to January 15, 2019), there were 2,267 unique inpatient visits with 0.603 BMP/CMP/RFP and 0.479 CBC/CBCPD results per patient day representing a significant decrease of 9.3% and 14.5% change from baseline for BMP/CMP/RFP and CBC/CBCPD orders, respectively ($p < 0.0001$ for each). Combined, an overall 11.7% decrease in laboratory testing was achieved ($p < 0.0001$) with an estimated cost savings of $398,884 in patient charges and 10,214 mL in blood. Despite significant reduction in laboratory testing, there were no statistically significant changes in the mortality rate, ICU transfer rate, and average hospital length of stay compared to the baseline period. There were no significant difference in the average Elixhauser Comorbidity Index or case mix index in the pre- and post-intervention periods.

Conclusions: After 2.5 months, this study demonstrates that interventions via email, verbal reports, and an online dashboard effectively reduce routine daily lab test ordering with no significant increase in adverse patient outcomes.

B-062

Performance Evaluation of Total PSA and free PSA Assays on the Atellica IM 1600 and Abbott Architect

P. P. Miranda, C. M. M. Dias, D. P. Aguiar, S. G. R. Santos. *DASA, Recife, Brazil*

Background: Total and Free Prostate Specific Antigen (PSA) assays are indicated, with the Digital Rectal Exam (DRE), helping to diagnose prostate cancer. In Brazil, prostate cancer is the most common type of cancer among men, after skin cancer, causing the death of 28.6% of those who develop this neoplasm. Free PSA is indicated for total PSA values between 4.0 and 10.0 ng/mL to differentiate between benign hyperplasia and prostate cancer (CA). In addition to differentiation, it helps guide the decision of a prostate biopsy. Given the importance of a reliable outcomes, the primary objective of this study was to demonstrate the analytical performance of the two trials between the Atellica IM 1600 Siemens Healthineers and Abbott Architect Plus i400sr platforms and to assess the impact on methodology change in patient follow-up. Studies included imprecision verification and methods comparison. **Methods:** In order to evaluate the imprecision, a study of repeatability (%CVR) and run-to-run variation (%CVWL) was performed for the fPSA and total PSA assays on the Atellica IM 1600 Analyzer according to EP15-A3, and the methods comparison according to EP09-A3. For the imprecision study, three concentrations were used; each level of QC materials was tested in one run per day, with five replicates per run, for five days, resulting in a total of 25 replicates per sample for each assay. Method comparison studies were performed with the Atellica IM 1600 and Architect Plus i400sr Analyzer assays using 26 serum samples that covered every linearity range. **Results:** The imprecision results are consistent with the manufacturer's claims. The %CVR was from 1.212% to 1.643% and the %CVWL were 2.504% to 3.821% among all the Atellica IM Analyzer assays. The assays tested on the Atellica IM Analyzer and Architect Plus i400sr demonstrated excellent agreement for fPSA ($R^2 = 0.9916$) and regression $Y = 0.8282 * x + (0.008)$, and for PSA ($R^2 = 0.9986$) and regression $Y = 0.844 * x + (0.025)$ and the graph of relative differences showed that > 95% are within the total error calculated for each difference**.** **Conclusion:** The assays tested on the Atellica IM 1600 analyzer demonstrated adequate results, according to the specifications. The imprecision results are consistent with the manufacturer's claims, demonstrating an analytical performance capable of accurately measuring the prostate specific antigen and equivalent to the results of the previously used laboratory platform, minimizing patient impact and assisting in the diagnosis and monitoring of prostate cancer.

B-063

Standardization of Chemistry Reference Ranges (Adult) in a Health Network with Multiple Laboratories

Y. Trull. *Allegheny Health Network, Pittsburgh, PA*

Background: At Allegheny Health Network (AHN), adult chemistry reference ranges were in need of revision and standardization among 7 laboratories, which serve 5 hos-

pitals and 1 ambulatory service facility in the areas centered at Pittsburgh, PA. All the aforementioned laboratories use Roche chemistry (c501/c502/c311) and immunoassay (e602/e411) analyzers. The major disagreement in reference intervals came from the fact that some AHN laboratories adopted manufacturer's recommended ranges, while others were using inherited reference intervals.

Method: For tests commonly ordered in outpatient settings, such as BUN, creatinine, sodium, potassium, total bilirubin, ALT, AST, ALP, total protein and albumin, 200 de-identified adult patient results were extracted from laboratory information system based on ICD 10 codes. The codes are Z00.00, Z00.5, Z00.6, Z00.8, Z02.1, Z02.3, Z02.81, Z02.83, Z02.89, Z04.6, which represent relatively "healthy" population presented at AHN for physical examinations. For each analyte, results were first sorted from minimum to maximum and outliers were excluded by Chauvenet criterion. Then frequency distributions of results were plotted, in respective to genders and ages if needed. Hoffmann indirect method was employed to establish reference intervals when necessary. The existing reference intervals were compared to the frequency distribution plots or the established reference ranges, for acceptability based on reference change values.

For tests that are not commonly ordered with the ICD codes of outpatient physical examinations, such as CSF tests, existing reference ranges, together with published literatures were presented to medical directors of the AHN laboratories for discussion and consensus. For some therapeutic drug monitoring tests, pharmacy was consulted for their reference ranges.

Results: For tests like BUN, creatinine, sodium, total bilirubin, ALT, AST, ALP, total protein and albumin, the manufacturer's recommended reference ranges were either verified with the frequency distribution plots or found clinically comparable to the established ranges. For potassium, a range of 3.5 - 5.2 mmol/L was adopted, taking into consideration that serum potassium is on average 0.2 mmol/L higher than plasma potassium. For reference ranges of the tests that were determined by clinical consensus, the manufacturer's recommended ranges were generally adopted, with exceptions like vancomycin trough, which was decided to be 10.0 - 20.0 ug/mL.

Conclusion: This abstract describes a process to standardize chemistry reference ranges (adult) at AHN. The process is supported by both evidence-based study and clinical consensus.

B-064

Changes in Physician Order Sets Result in a Sustained Reduction of Unnecessary Testing

N. J. Rutherford[1], Z. Shajani-Yi[2], J. LeGrand[1], J. H. Nichols[2]. [1]*Vanderbilt University Medical Center, Nashville, TN,* [2]*Vanderbilt University School of Medicine, Nashville, TN*

Inappropriate clinical testing that has little potential for clinical benefit should be minimized. In order to address misutilization, clinical laboratories can monitor usage patterns of these tests and subsequently devise strategies to guide clinician ordering. Previously, we had reported the short-term outcomes of a joint educational and laboratory order set intervention that was enacted in 2015 to address the frequent ordering of hemoglobin variant tests on low-risk obstetric patients, inconsistent with the American College of Obstetricians and Gynecologists (ACOG) guidelines. We previously found that these orders were mostly driven by a standardized electronic order set that required clinicians to manually remove the test for low-risk obstetric patients - an action that was often overlooked in the clinical environment. As previously reported, this strategy resulted in a reduction of the number of hemoglobin variant tests ordered for low-risk obstetric patients to almost zero in the 2.5 months immediately following the implementation of these changes. Here, we report a follow-up study to assess if these changes remained effective in the long-term by reviewing ordering patterns four years later. Nine hundred and eighty hemoglobin variant orders from five clinics were reviewed for inappropriate testing of low risk obstetric patients. Due to limitations of available racial and ethnic data in the electronic medical record, low risk obstetrics patients were defined as non-Hispanic, non-Arab Caucasians with a normal MCV and hemoglobin concentration. Prior to the intervention, 24.9% of orders were identified as inappropriate. Post-intervention the number of inappropriate orders decreased to 5.4%. After four years the number of inappropriate orders remained similar at 4.8%. As expected, there was a shift in the demographics of patients undergoing hemoglobin variant testing before versus after the intervention, with a decrease in the number of patients who identified as non-Hispanic, non-Arab Caucasian (36.2% vs 13.5%). Interestingly, there was also a marked increase in the number of patients who declined to report their race in 2018 compared to 2014-2015 (25.5% vs 6.52%) that may be a confounder of the long-term results. The results of this study demonstrate the effectiveness of monitoring and strategically curating clinical order sets to encourage better compliance with institutional or societal guidelines for patient testing.

B-065

Strategies in Safety: Improvementof Patient/Specimen Identification Errors

M. CUADRADO CENZUAL, L. Collado, E. Martinez, M. Cárdenas, a. Santiago. *HOSPITAL CLINICO SAN CARLOS, MADRID, Spain*

Background: Patient Identification and specimen labeling represent one of the most critical area in patient safety and is an increasingly visible mission for clinical laboratories *.The aim of this work is* to assess patient identification and specimen labeling improvement after implementation projects using longitudinal statistical tools

Methods: Patient/Specimen Identification errors were categorized by a multidisciplinary health care team. They were grouped into 3 categories: A: specimen/requisition mismatch, B: unlabeled Patient Identifications, C: Misidentification Patient. These types of identification errors were compared preimplementation and postimplementation for 3 patient safety projects: 1: Developpment of Identification Patient and Specimen Process to follow by all the professionals implied. (2) reorganization of phlebotomy ; (3) introduction of an electronic event reporting system. We use trend analysis and Student t Test

Results: Of 52632 total requests analyzed, requisition mismatches, unlabeled patient Identification and misidentification patient ; represented 1.4/10,000, 5.8/10.000, and 4.0/10.000of errors, respectively. Student ttest showed a significant decrease in the two most serious errors: mislabeled specimens (P< .001) and Misidentification Patient (p< .001) when compared to before implementation. Trend analysis demonstrated decreases in all 3 error types for 18 months.

Conclusion: The applied strategies have demonstrated to be effective in the improvement of the identification of the patient in the analytical requests. However, we must continue working in this strategy, with all the implied professionals and trying to reach the objective of which the 100% of the requests they are identified correctly

B-066

Dose-Dependent Effect of Pyruvate on Alanine Aminotransferase Activity Measured on Ortho's Vitros Dry Slide Technology

M. Ali[1], S. Devaraj[2]. [1]*Department of Pathology, SUNY Upstate Medical Hospital, Syracuse, NY,* [2]*Department of Pathology & Immunology, Baylor College of Medicine/Texas Children's, TX*

Background: Recently, Ortho-Clinical Diagnostics (Ortho) launched the new VITROS® ALTV Slides, which utilizes dry slide technology. At Texas Children's Hospital immediately after implementation of the new slides on Vitros 5600, they were multiple encounters where the ALT samples triggered "DP" error message. Upon investigation, the manufacturer's package insert mentions that higher pyruvate levels may trigger DP (Substrate depletion) flag. Till date, there is no study about the interference of pyruvate on ALT assays. Herein, we investigated the effect of dose-dependent concentrations of pyruvate on ALT, measured using Ortho's Vitros dry slide technology at our pediatric hospital.

Methods: We evaluated the effect of varying concentrations of pyruvate (0.005-0.04mg/ml) on high and low values of ALT pooled using de-identified serum samples. We measured ALT enzyme activity both on unspiked low and high ALT serum pools, as well as pyruvate spiked low and high ALT serum pools on Vitros 5600 analyzer to investigate significant bias in the spike samples compared to unspiked samples in the quantitative immunoassay.

Results: Our data suggest that for lower ALT values, pyruvate up to 0.02 mg/ml concentration resulted in <10% positive bias and pyruvate >0.02mg/ml concentration resulted in DP error message. Subsequently, on the higher ALT values, pyruvate up to 0.08mg/ml has <10% negative bias.

Conclusion: In conclusion, the 'low-normal' ALT values are of no significance. The 'high abnormal' ALT values may show falsely low or normal due to endogenous pyruvate, so on accounts of unexpectedly high or low ALT values, an appropriate investigation should be performed so the providers are aware of such interferences. Therefore, we strongly recommend the samples with DP error message be repeated following dilution. Fortunately, the manufacturers have changed the reagent formulation and have corrected the substrate depletion error with their re-formulated assay.

Wednesday, August 7, 2019

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

Mass Spectrometry and Separation Sciences

B-067

Validation of an UPLC-MS/MS Method to Simultaneously Measure Four Immunosuppressant Drugs with Deuterated Internal Standards

S. Yun[1], Y. Cho[1], S. Shin[2]. *[1]Korea university medical center, Seoul, Korea, Republic of, [2]Seoul Metropolitan Dongbu Hospital, Seoul, Korea, Republic of*

Background: Whole blood concentrations of immunosuppressant drugs such as cyclosporine A (CsA), tacrolimus (TAC), sirolimus, and everolimus should be monitored to minimize the risk of rejection and side effects of the drug. Ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) is recommended for immunosuppressant drug analysis due to their high specificity in comparison to immunoassay performance. In this study, we developed and validated a UPLC-MS/MS method for the simultaneous measurement of four immunosuppressant drugs in whole blood samples.

Methods: The UPLC-MS/MS method was developed for simultaneous determination of CsA, TAC, sirolimus, and everolimus in whole blood and the accuracy, precision, limit of quantification (LOQ), matrix effect, and stability were validated. The UPLC-MS/MS method was compared with the chemiluminescence immunoassay (CLIA) and enzyme immunoassay (EIA) methods for the 884 TAC and 298 CsA clinical samples.

Results: The inaccuracy and imprecision with the four QC levels were −7.7% to 2.6% and <9.0% for CsA, −6.9% to 4.7% and <8.9% for TAC, −8.8% to 6.1% and <7.8% for sirolimus, and -7.1% to 11.6% and <16% for everolimus, respectively. The LOQs were 0.5 ng/ml for TAC and sirolimus, 1.0 ng/ml for everolimus, and 10 ng/ml for CsA. Interference from the matrix and ion-suppression at their elution times were not observed. CsA, TAC, sirolimus, and everolimus were stable until 40 days at -20 °C with an inaccuracy ranging from -14.1% to 16.2%, -12.0% to 18.3%, -12.0% to 13.4% and -0.3% to 12.3%, respectively. The correlation coefficients between the immunoassay and UPLC-MS/MS were 0.9689 for CsA and 0.9529 for TAC, respectively. The mean overestimation of the immunoassay, as compared to UPLC-MS/MS was 9.6% (-35.4% to 82.0 %: 95% limit of agreement) for CsA and 9.6% (-24.1% to 54.9% : 95% limit of agreement) for TAC, respectively.

Conclusion: We developed and validated a fast and robust UPLC-MS/MS method for routine therapeutic drug monitoring and testing of four immunosuppressants simultaneously.

B-068

Validation of a SPME-GC-MS/MS Method for Measuring Homovanillic Acid and Vanillylmandelic Acid on a Thermo Scientific TSQ Duo Tandem Mass Spectrometer and Comparison to a LC-MS/MS Method

K. J. Wickware, E. Weinzierl, V. Leung-Pineda. *Children's Healthcare of Atlanta, Atlanta, GA*

Background: Homovanillic acid (HVA) and vanillylmandelic acid (VMA) are urinary byproducts of catecholamine metabolism which are elevated in the clinical setting of neural crest tumors such as neuroblastomas. The quantitation of these analytes in urine is instrumental in quickly initiating treatment of patients with suspected neuroblastoma. The standard method for analysis at reference laboratories is LC-MS/MS. As our laboratory did not have access to LC-MS/MS, we developed a method using GC-MS/MS. In place of the traditional liquid-liquid extraction and BSTFA derivatization commonly used for GC-MS methods, which are time-consuming and require multiple manual steps, we validated a method for solid-phase microextraction (SPME). The advantages of the SPME method are simple sample preparation and no introduction of organic solvents into the column or system.

Objective: Our goal was to determine the performance specifications of a SPME-GC-MS/MS method for measuring HVA and VMA, and to compare the method to an established LC-MS/MS method.

Methodology: The following parameters were evaluated to validate the SPME-GC-MS/MS method: Linearity, lower limit of the measuring interval (LLMI), matrix effect, carryover, accuracy, dilution, precision and interferences.

Results & Conclusions: Linearity was determined to be 0.1 - 4.0 mg/dL for HVA and 0.1 - 5.0 mg/dL for VMA; dilution enabled measurement up to 20 mg/dL and 25 mg/dL, respectively. No issues due to matrix effects were detected. Carryover was determined to be less than 0.5%. There was no clinically significant interference due to presence of high concentrations of hemoglobin, bilirubin or creatinine. Inter-day and intra-day precision studies conducted at two different concentrations (low and high) yielded CVs < 8%. Accuracy was assessed by method comparison using patient samples and spiked samples to an established reference LC-MS/MS method (n=50); comparison to the reference method yielded the following statistics: For HVA, slope = 1.022, intercept = -0.08668, R^2 = 0.9926; for VMA, slope = 1.013, intercept = -0.04494, R^2 = 0.9825. Proficiency testing (PT) events were also utilized to assess the method; PT results were within 0.8, 0.6 and 0.7 SDI of the group mean for HVA and 0.2, 1.1 and 0.9 SDI of the group mean for VMA. Also of note, sample preparation time due to use of SPME was reduced from about four hours to approximately 45 minutes compared to a traditional GC-MS method. The performance characteristics of our SPME-GC-MS/MS method were shown to be clinically comparable to a LC-MS/MS method.

B-069

A Novel Photo-Sensitive Nanoprobe Combined with Mass Spectrometry for Quantitative Detection of PD-L1

Y. Chen, J. Zhu. *Nanjing Medical University, Nanjing, China*

Background: To date, therapies that inhibit checkpoint of programmed cell death 1 (PD-1) and its ligand programmed death ligand 1 (PD-L1), have shown unprecedented rates of durable clinical responses in patients with various cancers. In addition, much evidence has demonstrated that PD-L1 expression status is an important indicator of prognosis and therapy responsiveness. Current PD-L1 detection in clinical practice primarily relies on immunohistochemistry (IHC) analysis, whereas the scoring system and cut-off values vary among individual studies. These will lead to a more complicated PD-L1 testing process and result in inappropriate treatment decisions. Therefore, a method that can quantitatively detect the expression level of PD-L1 in tumors was on demand. In this work, an assay combining a novel photo-sensitive nanoprobe and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed.

Methods: In the assay, we converted the PD-L1 signal to the mass response of a reporter peptide. The photo-sensitive nanoprobe consisted of a photo-cleavable amphiphilic peptide (AVLGDPFR-photo-sensitive linker-C18) and a specific PD-L1 aptamer. This nanoprobe could specifically bind to PD-L1 on cell surface and release the reporter peptide under UV-light irradiation. Finally, the amount of the reporter peptide, representing the PD-L1 level, could be determined by LC-MS/MS.

Results: The prepared nanoprobe has a small size (63 ± 1.3 nm), and it could quickly disassemble under UV-light irradiated for 2 h. Its binding affinity (Kd value) was 202.4 nM. Moreover, the stability, binding specificity and photo-cleavable efficiency of this novel nanoprobe were characterized. The resulting lower limit of the measuring interval (LLMI) for PD-L1 was 20 pmol/L, and the detection dynamic range spanned about 2 orders of magnitude from 20 to 2000 pmol/L. Both accuracy and precision were ≤ 15%. Finally, the assay was employed to determine the PD-L1 level in several breast cancer cell lines including MDA-MB-231, MDA-MB-468, MCF-7 and T47D, and 80 pairs of human breast primary tumors and normal tissue samples. The result was highly concordant with that obtained by IHC (κ = 0.870), whereas the possibility of false-positive findings detected by IHC was also indicated.

Conclusion: The novel photo-sensitive nanoprobe combined with LC-MS/MS can provide the PD-L1 values in a more quantitative manner and can easily be used for reference range establishment and further substratification. However, significant efforts are still required to make it as applicable as those approved assays.

B-070

A Novel Photo-Sensitive Nanoprobe Combined with Mass Spectrometry for Quantitative Detection of PD-L1

Y. Chen, J. Zhu. *Nanjing Medical University, Nanjing, China*

Background: To date, therapies that inhibit checkpoint of programmed cell death 1 (PD-1) and its ligand programmed death ligand 1 (PD-L1), have shown unprecedented rates of durable clinical responses in patients with various cancers. In addition, much evidence has demonstrated that PD-L1 expression status is an important indicator of prognosis and therapy responsiveness. Current PD-L1 detection in clinical practice

primarily relies on immunohistochemistry (IHC) analysis, whereas the scoring system and cut-off values vary among individual studies. These will lead to a more complicated PD-L1 testing process and result in inappropriate treatment decisions. Therefore, a method that can quantitatively detect the expression level of PD-L1 in tumors was on demand. In this work, an assay combining a novel photo-sensitive nanoprobe and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed.

Methods: In the assay, we converted the PD-L1 signal to the mass response of a reporter peptide. The photo-sensitive nanoprobe consisted of a photo-cleavable amphiphilic peptide (AVLGDPFR-photo-sensitive linker-C18) and a specific PD-L1 aptamer. This nanoprobe could specifically bind to PD-L1 on cell surface and release the reporter peptide under UV-light irradiation. Finally, the amount of the reporter peptide, representing the PD-L1 level, could be determined by LC-MS/MS.

Results: The prepared nanoprobe has a small size (63 ± 1.3 nm), and it could quickly disassemble under UV-light irradiated for 2 h. Its binding affinity (Kd value) was 202.4 nM. Moreover, the stability, binding specificity and photo-cleavable efficiency of this novel nanoprobe were characterized. The resulting lower limit of the measuring interval (LLMI) for PD-L1 was 20 pmol/L, and the detection dynamic range spanned about 2 orders of magnitude from 20 to 2000 pmol/L. Both accuracy and precision were ≤ 15%. Finally, the assay was employed to determine the PD-L1 level in several breast cancer cell lines including MDA-MB-231, MDA-MB-468, MCF-7 and T47D, and 80 pairs of human breast primary tumors and normal tissue samples. The result was highly concordant with that obtained by IHC (κ = 0.870), whereas the possibility of false-positive findings detected by IHC was also indicated.

Conclusions: The novel photo-sensitive nanoprobe combined with LC-MS/MS can provide the PD-L1 values in a more quantitative manner and can easily be used for reference range establishment and further substratification. However, significant efforts are still required to make it as applicable as those approved assays.

B-072

Analysis of Vitamin A and Vitamin E on a Multiplexing LC-MS/MS Platform for Therapeutic Drug Monitoring (TDM)

L. Zha, T. Law, C. MacDonald, R. W. A. Peake, M. D. Kellogg. *Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA*

Background: Vitamin A and vitamin E are fat-soluble antioxidants routinely monitored to assess nutritional status in children, especially in patients of short bowel syndrome, celiac disease, and other gastrointestinal disorders. Typically clinical laboratories measure these analytes using HPLC. Successful implementation of MS-based testing requires laboratories to set up assays on a limited number of systems. To address this challenge and streamline the workflow, we report the integration of quantitative vitamin A and E analysis into a single multiplexing LC-MS/MS platform already measuring vitamin D, busulfan, antiepileptic drugs, and immunosuppressant drugs.

Methods: LC-MS/MS analysis of sample extracts containing internal standards (retinol-d_6 and α-tocopherol-d_6) was performed on a TLX-2 online sample preparation liquid chromatography (SPLC) system (Thermo-Scientific) coupled to an API5500 Q-Trap tandem mass spectrometer (ABSciex). The two channels on the SPLC were each equipped with a TurboFlow™ column for sample clean-up and an analytical column. Vitamin A, E, and D were analyzed on channel 1, while all therapeutic drugs were monitored using channel 2.

Assay imprecision was determined by replicate analysis (n = 20) at three levels over 16.0-103.8 µg/dL vitamin A and 4.9-28.3 mg/L vitamin E. The lower limit of quantification (LLOQ) was assessed by replicate measurement (n = 10) of calibrators. The performance of the LC-MS/MS method was compared to the current HPLC method by measuring patient specimens (n = 20) over 11-67 µg/dL vitamin A and 1-17 mg/L vitamin E. Interference was assessed by utilizing commercial multi-level TDM products.

Results: Linearity of vitamin A and E (r > 0.999) was established over the range of 0-135.0 µg/dL for vitamin A (LLOQ = 9.4 µg/dL, CV = 2.3%) and 0-27.9 mg/L for vitamin E (LLOQ = 0.9 mg/L, CV = 2.9%). At low, medium, and high levels, the CV of the assay were 2.5, 2.9, and 4.3% for vitamin A and 5.0, 4.4, and 6.9% for vitamin E. Deming-regression between the LC-MS/MS method and the current HPLC method revealed (Slope and Intercept) S = 0.930 ± 0.036, I = 2.0 ± 1.4, P < 0.0001 for vitamin A and S = 0.951 ± 0.050, I = 0.08 ± 0.28, P < 0.0001 for vitamin E. Preliminary assessment indicated the assay was not interfered by common therapeutic drugs.

Conclusions: Our study established that the LC-MS/MS method is suitable for the analysis of vitamin A and E in a clinical laboratory. Expansion of the test menu on a multiplexing platform has the potential to further reduce instrument downtime and improve the testing capability and throughput of our clinical laboratory.

B-073

Quick Multiplexed Analysis of Cannabinoids and Their Metabolites in Urine using MassHunter StreamSelect LC-MS System

A. Szczesniewski, K. McCann. *Agilent, Wood Dale, IL*

Introduction: Liquid chromatography triple quadrupole mass spectrometry (LC/MS/MS) is the ideal solution for the simultaneous analysis of multiple cannabinoids and metabolites due to the high specificity and sensitivity of the instrumentation. A chromatographic method was developed to separate following analytes - cannabidiol (CBD), cannabidiolic acid (CBDA), cannabinol (CBN), tetrahydrocannabinol (THC), nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), 11-hydroxy-Δ9-tetrahydrocannabinol (THC-OH) - using an Agilent 1290 Infinity II Liquid Chromatograph. Quantitative data was acquired using an Agilent's 6470 triple quadrupole mass spectrometer. Sample throughput was nearly quadrupled (3.7x) by running four simultaneous, staggered chromatographic analyses on a single mass spectrometer using Agilent's MassHunter StreamSelect LC-MS software.

Method: Instrumentation includes an Agilent 6470 Triple Quadrupole LC/MS with Agilent JetStream (AJS), four 1290 Infinity II High Speed Pumps, four 1290 Infinity II Multicolumn Thermostats, and a StreamSelect RSI (PAL3) Autosampler. A 4.4 minute gradient (water:methanol, 0.01% formic acid, 5mM ammonium formate) with a 1.6 minute equilibration was run on an Agilent Poroshell 120 EC-C18 column. Quantitation was performed with optimized MRM transition pairs for each analyte and internal standard in positive mode. Urine was spiked with 6 cannabinoids and serially diluted to get calibration curve, (range 5 - 5000 ng/mL). A batch of 1000 identical samples were made at 100 ng/mL. Calibrators and samples were diluted 1:10 with 70% methanol containing labeled internal standards at 50 ng/mL.

Preliminary Data: A 1/x weighting factor was applied during linear regression of the calibration curves. The quantitation using chromatographic peak area ratio to a known concentration of the internal standards. Each analyte was quantitated with its own deuterated internal standard, expect for CBDA, which was quantitated with CBD-d3. Samples and calibrators were grouped and quantitated based on the stream on which they were acquired. All calibration curves displayed excellent linearity. Retention time reproducibility between all four chromatographic streams was between 0.43% and 1.54% (n = 1000), depending on the analyte. StreamSelect acquired data for 894 samples over a period of 24-hours, which equates to 97 seconds per analysis. Compared to a 6-minute runtime for the same analysis using traditional LC-MS, this results in a 3.7x increase in sample throughput. Over the course of nearly 27 hours, 1000 identical urine samples containing 100 ng/mL of each of the six analytes and their respective internal standards were run across the four LC streams. Quantitative reproducibility was consistent across all streams.

Novell Aspect: Full, robust chromatographic separation and LC/MS analysis of 6 cannabinoids at a rate of 97 second per sample.

For Research Use Only. Not for use in diagnostic procedures.

B-074

Development and Clinical Validation of Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Assay for Quantitation of Creatinine in Dried Blood Spots

B. Y. Owusu[1], C. Gordon[2], K. Sadilkova[2], J. A. Dickerson[2]. [1]*University of Washington, Seattle, WA,* [2]*Seattle Children's Hospital, Seattle, WA*

Background: Clinical analysis of creatinine (Crn) is useful not only for monitoring creatinine levels but also for optimizing therapeutic doses of nephrotoxic and renally excreted drugs. Dried blood spot (DBS) creatinine measurement by LC-MS/MS would serve as an alternative method for currently used plasma enzymatic creatinine assay. This would also enhance the clinical utility of remote collection of patient DBS for therapeutic drug monitoring. The use of DBS for therapeutic drug monitoring of immunosuppressants has been developed and is currently being utilized here at Seattle Children's Hospital (Sadilkova et al., 2013; Dickerson et al., 2015).

Objective: The objective of this research was to develop and validate a LC-MS/MS method for the quantitative analysis of creatinine in patient-collected DBS extracts and to compare this method with the clinical routine enzymatic plasma creatinine method by Vitros 4600.

Methods: Capillary blood collected from finger-pokes and residual venous whole blood samples spotted on Whatman 903 filter paper were included when there was a paired plasma creatinine measured by Vitros 4600, collected within 24 hours of each other. The method was validated using a six-point calibration curve using 3-mm DBS extracts prepared from whole blood samples spiked with creatinine. Creatinine-$^{13}C_3$,d_3 was used as an internal standard and DBS creatinine levels were measured in the

positive multiple reaction monitoring (MRM) mode by Waters Xevo TQ-MS tandem mass spectrometer coupled with Waters Acquity UPLC. Chromatographic separation on AQUITY UPLC HSS T3 column was achieved using a mobile phase consisting of 2 mM ammonium acetate with 0.1% formic acid in water (mobile phase A) and 2 mM ammonium acetate with 0.1% formic acid in methanol (mobile phase B). The MRM transitions used for DBS creatinine quantification were m/z 114 > 86 (Crn) and m/z 120 > 91 (Crn-$^{13}C_3$,d_3). Qualification MRM transitions were m/z 114 > 44 (Crn) and m/z 120 > 49 (Crn-$^{13}C_3$,d_3).

Results: The assay was linear between 0.2 to 10 mg/dL. Inter-assay precision showed a coefficient of variation of 8.3% and 4.9% for low and high controls, respectively. Both capillary and venous whole blood DBS creatinine measured by LC-MS/MS correlated well with plasma creatinine measured by Vitros 4600. The correlation coefficient (R) of capillary DBS creatinine versus Vitros 4600 plasma creatinine was 0.98 (n=39, slope=0.94 and intercept 0.08) using Deming regression analysis. Spotted venous DBS creatinine versus Vitros 4600 plasma creatinine had a correlation coefficient of 0.99 (n=37, slope=1.00 and intercept 0.12). The limit of blank and limit of quantification were determined to be 0.0009 mg/dL and <0.1 mg/dL respectively. No carry-over was observed up to 10 mg/dL. Creatinine in the DBS samples was stable for at least 2 years at 4 °C and -70 °C. At RT, 37 °C and 60 °C, DBS creatinine was stable for at least 4 days.

Conclusions: Quantitative measurement of creatinine in capillary DBS by LC-MS/MS was shown to be reliable and could be used as an alternative method for plasma creatinine measurement by Vitros 4600. This will facilitate the clinical utility of remote collection of patient DBS for therapeutic drug monitoring.

B-075

Simultaneous Determination of Busulfan and Melphalan in Saliva by Turbulent Flow Chromatography Tandem Mass Spectrometry

D. Carlow, H. Landau, S. Giralt, G. Shah, M. Scordo, L. Ramanathan, M. Pessin, R. Schofield. *Memorial Sloan Kettering Cancer Center, New York, NY*

Background: Busulfan (Bu) and melphalan (Mel) are cytotoxic DNA alkylating agents that are used in many hematopoietic stem cell transplant (HCT) conditioning regimens. Taste disturbance, or dysgeusia, is commonly observed after HCT for patients with hematologic malignancies and is potentially related to drug levels present in saliva. Despite its frequency, dysgeusia has received limited prospective study, in part due to the lack of sensitive assays for these compounds that have been validated in saliva.

Objective: The objective was to develop a rapid and simple assay for the simultaneous measurement of Bu and Mel in saliva by turbulent flow liquid chromatography tandem mass spectrometry (TFLC-MS/MS) that has sufficient analytically sensitivity and a clinically useful dynamic range to detect low drug concentrations in saliva. This assay will be used to determine if there is a correlation between the drugs concentrations in saliva and the reported levels of dysgeusia in patients receiving these medications and to facilitate pharmacokinetic studies.

Methods: Bu and Mel were isolated from saliva samples (100 µL) after protein precipitation with methanol containing deuterated internal standard (Bu-d_8 and Mel-d_8) followed by centrifugation. The supernatant was injected into the turbulent flow liquid chromatography system followed by electrospray positive ionization tandem mass spectrometry (TFLC-MS/MS). Chromatographic separation was performed on a Thermo Scientific TLX-2 HPLC system interfaced to a TSQ Vantage mass spectrometer operated in positive ion ESI mode. MRM transitions were as follows: Busulfan 264.0>151.1

and 264.0>55.1 m/z; Melphalan 305.1>246.1 and 305.1>288.0 m/z. The results were quantified using a six-point calibration curve. Assay accuracy was determined through recovery studies. Within day imprecision was evaluated by analysing 10 specimens in a single day, and between-day imprecision was evaluated by running two batches in duplicate over 20 days.

Results: The LOQs of Bu and Mel were both 10 ng/mL; the CVs were <20% over 20 days and the signal to noise ratio was > 20:1. For Bu and Mel the calibration curves were linear over the analytical measurement range (AMR) with correlation coefficients $R^2 \geq 0.99$. Dilutions were validated giving a final clinical reportable range (CRR) of 10 to 25,000 ng/mL. Within day and between-day imprecision (CVs) at concentrations spanning the AMR were less than 6% for both analytes. Bu and Mel were sufficiently stable when placed on ice under all relevant analytical conditions. No significant matrix effects were observed during the method validation. Recoveries ranged from 95.6-106.2 for three controls spanning the AMR for both compounds.

Conclusion: We have developed and validated an accurate and sensitive TFC-LC-MS/MS method for the simultaneous quantification of busulfan and melphalan in human saliva. TFC for analyte extraction allows for significantly reduced sample preparation time, high throughput and small sample volume (100 µL). The method has been fully validated for imprecision, accuracy, linearity, recovery, carryover, specificity and matrix effects. This is the first reported method that simultaneously measures busulfan and melphalan in saliva, and due to the assays performance, it will be used to support clinical trials and pharmacokinetic studies.

B-076

Measurement of Symmetric Dimethylarginine by LC-MS/MS for the Evaluation of Renal Function

E. Wagner, D. Chu, K. Mercado, D. Fattore, C. Zipperle, E. Fix, S. Fischer, M. Militello. *Covance Central Laboratory Services, Indianapolis, IN*

Background: Symmetric and asymmetric dimethylarginine (SDMA and ADMA) are metabolites of the post-translational modification of arginine residues in histones. SDMA is produced by all body tissues and is cleared almost exclusively by the kidneys, making it an attractive biomarker for the evaluation of renal function. In this study, the development of an LC-MS/MS method for the measurement of SDMA is described and its performance reviewed against traditional renal markers in individuals with microalbuminuria or abnormal thyroid function.

Methods: Serum and K2-EDTA plasma were collected from consented normal donors. Remnant specimens from individuals with microalbuminuria or abnormal TSH values were de-identified per standard operating procedures. SDMA, arginine, ADMA, and internal standard SDMA-d6 were extracted from specimens by protein precipitation and dilution. Calibrators were prepared from spiked charcoal-stripped K2-EDTA human plasma with SDMA. SDMA and its internal standard were analyzed using Waters UPLC system (Milford, MA) coupled to an AB Sciex QTRAP 5500 mass spectrometer (Washington, D.C.) in multiple reaction monitoring (MRM) mode. Chromatographic separation was performed using Phenomenex normal phase column (Torrance, CA). The mobile phases consisted of 1% formic acid in water (phase A) and 50:50 methanol: acetonitrile (phase B). Phase A was adjusted 15% to 60% and phase B from 85% to 40% from 4.1 to 4.8 minutes before returning to initial conditions. SDMA and its internal standard were detected by positive electrospray ionization with the following transitions: m/z 203→172 and internal standard m/z 209→175.

Cystatin C was measured on a Siemens Behring Nephelometer II (Malvern, PA). Thyroid stimulating hormone (TSH) was measured on the Beckman Coulter Access DxI (Brea, CA). Creatinine was measured on the Roche cobas 8000 (Indianapolis, IN) using enzymatic and Jaffé reagent systems. Quality control (QC) samples were assayed prior to SDMA, cystatin C, creatinine, and TSH measurements.

Results: The LC-MS/MS method for SDMA is linear from 10 to 1000 ng/mL. Intra-day and inter-day precision is <3.2% and <6%, respectively. Accuracy studies by spike-and-recovery yielded recoveries from 89-99%. Specimen dilution was verified up to eight-fold. The reference interval was verified at 73.0 - 125.1 ng/mL for adult males and females. Specimen stability was established for up to 7 days ambient (20-25° C) and refrigerated (2-8° C) temperatures and up to 12 months for -70° C. Freeze-thaw stability was established for 5 cycles at -70° C and 3 cycles at -20° C. No interference was observed for hemolyzed, icteric or lipemic samples.

Mean creatinine, cystatin C, and SDMA values were statistically significantly different (p<0.0001) between normal donors and individuals with microalbuminuria. Mean creatinine values were statistically significantly different (p<0.01) between hyperthyroid (TSH<0.1 µIU/mL) and hypothyroid (TSH >10 µIU/mL) individuals. Cystatin C and SDMA showed no statistically significant difference in these individuals (p>0.05).

Conclusions: SDMA measurement by LC-MS/MS provides an analytically robust alternative to creatinine for the evaluation of renal function. Like creatinine and cystatin C, SDMA was elevated in individuals with microalbuminuria. Unlike creatinine, SDMA measurement was unaffected by thyroid status.

B-077

Development and Validation of a Liquid Chromatography Tandem Mass Spectrometry Method for Simultaneous Quantification of Salivary Melatonin and Cortisol

S. Shin, J. Oh, H. Oh, E. Joo, S. Lee. *Samsung Medical Center, Seoul, Korea, Republic of*

Background: Analysis of melatonin and cortisol, have been proved to be clinically important for diagnosis of sleep disorders and mood disorders. While the measurement of melatonin and cortisol in salivary samples has primarily relied on immuno-

assays, they suffer from interference, and lack specificity. Here we developed and validated a simple and rapid LC-MS/MS method for simultaneous measurement of melatonin and cortisol in human saliva samples.

Methods: Melatonin, cortisol, and labeled internal standards (d4-melatonin and d4-cortisol) were extracted with liquid-liquid extraction using 300 µL of saliva sample.

The LC-MS/MS method was developed on Agilent 6490 tandem mass spectrometer equipped with Agilent 1260 HPLC system (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was done on C18 2.1 mm×50 mm 2.6 µm Kinetex column, with mobile phase consisting of a 2 mM ammonium acetate in DW and 0.1% (v/v) formic acid in acetonitrile. The analytes were detected in multiple-reaction monitoring mode by using electrospray ionization: melatonin, m/z 233.1→174.1; d4-melatonin, m/z 237.1→178.1; cortisol, m/z 363.1→120.9; d4-cortisol, m/z 367.4→121.1.

A 5-point calibration curve was prepared with stock solution for melatonin and cortisol diluted with methanol and 0.04% ascorbic acid. Three levels (2, 20, 80 pg/mL for melatonin; 0.2, 2, 8 ng/mL for cortisol) of quality control samples were analyzed on everyday of analysis.

Our LC-MS/MS method was validated according to CLSI guidelines (C50-A, C62-A) including linearity, precision, accuracy, recovery, matrix effect, limit of quantification, selectivity and carryover. In addition, method comparison was performed between our LC-MS/MS method and ELISA (Direct Salivary Melatonin Elisa EK-DSM. Bühlmann Laboratories AG.Schönenbuch, Switzerland) for melatonin and ECLIA (Cortisol II, Roche, Germany) for cortisol, using 120 saliva specimens from volunteers with IRB approval and informed consents.

Results: The chromatographic run time was 6 minutes. Calibration curves were linear over the calibration range for melatonin (0.5 - 100 pg/mL) and cortisol (0.05-10 ng/mL), with R^2>0.99. Intra-assay (n=5) and inter-assay (n=5) imprecisions were CV< 6% for both analytes at three levels. The lower limit of quantificaiton was 0.5 pg/mL for melatonin and 0.05 ng/mL for cortisol. Accuracy was < +/-10% of bias for both melatonin and cortisol at three levels. Recoveries for melatonin ranged from 100.9% to 102.6% and 100.1% to 103.7% for cortisol. No significant matrix effect was observed with values of 92.1% to 97.7% for melatonin and 98.8% to 99.0% for cortisol. No apparent carryover and interference were observed.

Melatonin levels measured with ELISA were higher than those of LC-MS/MS method with R^2 of 0.83, slope of 0.77 and intercept of 0.08. The mean bias was 23.16% (range -54.0%-143.7%). Cortisol levels measured with ECLIA were also higher than those of LC-MS/MS method with R^2 of 0.91, slope of 0.80 and intercept of -0.02. The mean bias was 48.9% (range -59.7%-184.7%).

Conclusion: Our LC-MS/MS method for simultaneous quantification of melatonin and cortisol demonstrates excellent analytical performance and short analytical time needed for clinical use. There was a significant and inconsistent difference in the results between our LC-MS/MS method and immunoassays for both analytes, suggesting inaccuracy of immunoassay method.

B-078

Accuracy of Three Total Testosterone Methods: ECLIA, RIA and LC-MS/MS

K. Robyak, C. Hamilton, P. McGhee, C. Pederson, M. Straub, S. Scott, T. Neibauer, W. Salter, M. Creer, Y. Zhu. *Penn State University, Hershey, PA*

Introduction: Total testosterone is currently measured by either immunoassay or high performance liquid chromatography tandem mass spectrometry (LC-MS/MS) in the clinical laboratory. Selection of the methodology must balance turn-around time, equipment cost, skilled personnel, accuracy, precision, specificity and sensitivity of the assay. Among these, accuracy is an important parameter of the performance characteristics of assays. The objective of this study was to determine the accuracy of our in-house assays including radioimmunoassay (RIA), LC-MS/MS, and electrochemiluminescence immunoassay (ECLIA) in comparison with a reference laboratory LC-MS/MS method.

Methods: Fifty patient serum samples with total testosterone range of 3 to 1500 ng/dL determined by a reference laboratory LC-MS/MS method were used in this study. The values of total testosterone concentrations were evenly distributed across the range. Specimens were analyzed by our in-house ECLIA on a Roche Cobas 8000 Chemistry System, RIA by MP Biomedicals Double Antibody method, and LC-MS/MS. Results of the in-house methodologies were compared with the reference laboratory values using the Alternate Quantitative Method Comparison program with Deming regression analysis (EP Evaluator, Data Innovations) and Microsoft Excel. The allowable total error limit is 25%.

Results: Compared to the reference laboratory LC-MS/MS method, all three in-house assays demonstrated a negative percent bias (mean ± SD): -11.2% ± 8.5% (LC-MS/MS), -8.0% ± 36% (ECLIA), and -40.2% ± 9.4% (RIA). The correlation between both LC-MS/MS (y_1) and ECLIA (y_2) with respect to the reference laboratory LC-MS/MS (x) was excellent: y_1 = 0.939x - 8.3 (R = 0.9978, SEE = 22.2 ng/dL, N = 50) and y_2 =0.899x + 3.1(R = 0.9948, SEE = 32.7 ng/dL, N = 47), respectively. Although the average bias between the in-house LC-MS/MS and ECLIA are similar (-11.2% vs -8.0%), the range of bias within the immunoassay is much wider, especially at lower concentrations. For samples with total testosterone concentrations less than 50 ng/dL, compared to the reference laboratory LC-MS/MS, the bias for ECLIA was -6.0 ± 53.5%, whereas it was -10.8% ± 11.6% for the in-house LC-MS/MS. The RIA method (y_3) showed the largest negative bias -40.2% ± 9.4%, y_3=0.36x + 25 (R = 0.9099, SEE = 60.8 ng/dL, N = 31).

Conclusions: Compared to the reference lab LC-MS/MS method, both in-house LC-MS/MS and ECLIA are agreeable and acceptable for patients with testosterone greater than 50 ng/dL; however, the LC-MS/MS results have a decreased variability of bias at lower testosterone concentrations (<50 ng/dL), which would be a distinct advantage for females, children, and hypogonadal males. Our data support that LC-MS/MS is the recommended technology for analysis of total testosterone for women, children, and men with hypogonadism. Therefore, we have selected LC-MS/MS as a reliable method for total testosterone testing. We also conclude that RIA has an unacceptable agreement with the reference laboratory LC-MS/MS method.

B-079

The Quantitation of Glycated Albumin by Isotope-Dilution LC-MS/MS

K. Kabytaev, A. Shin, S. Connolly, R. Little. *University of Missouri, Columbia, MO*

Background: Glycated albumin is considered an alternative glycemic indicator in certain situations where HbA1c does not accurately reflect glycemic status. These patient cases are usually associated with decreased erythrocyte lifespan, gestational diabetes or end-stage renal disease. Previously, we had developed an MS semi-quantitative method for glycated albumin (AACC, 2018). The current study is the logical next step in improvement of the method using an isotope-dilution approach.

Methods: The plasma from non-diabetic and diabetic subjects was extracted according to general clinical laboratory protocol. The samples were enzymatically digested by Glu-C with a preliminary reduction/alkylation step. The samples were then spiked with two isotope-labeled standards RQIKKQTALV(D_8)E and RQIKK(glycated) QTALV(D_8)E and analyzed on an LC-MS system with a linear gradient (water, acetonitrile, formic acid). Two MRMs (M^{3+}-> $(b_9\text{-}3H_2O)^{2+}$ and M^{3+}-> $(b_{10}\text{-}3H_2O)^{2+}$) were used for each peptide then the average value was calculated. The final results were presented as percentages of glycated/non-glycated RQIKKQTALVE. Glycated albumin was also measured in these samples using the commercially available Lucica GA-L (Asahi Kasei, Japan) method. **Results:** There was a clear linear correlation between our LC-MS/MS and the commercial Lucica GA-L method (Y=2.053*X) with a correlation coefficient of r^2 = 0.97. The CV for glycated albumin by LC-MS/MS was less than 1.3%.

Conclusion: We have developed a mass spectrometry-based absolute quantitative method for glycated albumin. The method includes a simple sample preparation and has demonstrated good reproducibility, linearity and precision. In the future, the developed method may be used as a reference method. To our knowledge, this is the first implementation of isotope dilution concept for enzymatically digested glycated albumin.

B-080

Validation of a One-Step Protein Extraction Method for a Multiplexed Steroid LC/MS Assay

A. Davis, C. MacDonald, T. Law, M. Kellogg. *Boston Childrens Hospital, Boston, MA*

Background: Steroid analysis in the BCH laboratory currently uses LC/MS/MS technology for its multiplexed steroid assay: Delta 4-Androstendione (Delta 4), Dihydroepiandrosterone (DHEA), Testosterone (Testo), and 17α- Hydroxyprogesterone (17 OHP). Although the current method provides accurate results, sample preparation is time consuming and poses a health risk: diethyl ether is used to solubilize proteins in specimens, and as a consequence, the specimens must be dried under vacuum and reconstitution in methanol before injection. The use of this method results in a 24-48 hr turnaround time (TAT) for results to be released to the provider, including requests to confirm a newborn screen positive for congenital adrenal hyperplasia. The new method described herein is a one step "dilute and shoot" protein extraction protocol

that not only removes the use of harmful chemicals, but also, reduces sample prep time to 20-30 min for a decreased TAT of <8 hrs.

Methods: The new extraction procedure consisted of mixing 150uL of extraction solution (Zinc sulfate in methanol with 10ng/mL of each deuterated steroid as an internal standard) and 150 uL of sample to be analyzed in an Eppendorf tube. Mixtures were vortexed for 10 seconds and centrifuged at 13,000 rpm for 10 min. Supernatant (200 uL) was transferred to glass vials for testing. Chromatographic separation was obtained using the Sigma-Supelco Titan C18 column (10cm x 3mm X 1.9um, Sigma). Mass spectrophotometry was performed using a Sciex 6500Q-Trap, TurboIonSpray source. Data was collected and analyzed using Analyst 1.6.2 software couple with Aria 1.7 TLX-2 LC system (Thermo Fisher). Simple precision was determined using plasma spiked with 2 concentrations (50 ng/mL and 350 ng/mL) of the respective analyte (n=20). Linearity (n=8) was analyzed over the current analytical range of 10 to 1500 ng/mL using an allowable systematic error (SEa) =7.5% for each analyte. Method comparison was performed (n=70) to determine equivalency between the current and new procedure.

Results: Simple precision for each concentration of analyte was acceptable with an observed CV of no more than 7.7% (50ng/mL DHEA). SEa for linearity throughout the current analytical range for each analyte was acceptable (17 OHP-SEa=7.3%; Delta 4- SEa=4.7%; DHEA- SEa=3.4%; and Testo- SEa=6.7%). Lastly, method equivalency was demonstrated for each analyte (17 OHP-slope=1.2, intercept=-2.77, R=0.988; Delta 4-slope=1.2, intercept = 0.054, R=0.988; DHEA-slope=1.05, intercept=-5.89, R=0.962; and Testo-slope=1.05, intercept =0.457, R=0.979).

Conclusion: The data obtained in this study demonstrated that the one-step protein extraction method produces acceptable performance for precision, linearity, and method comparison and supports the use of this method for more efficient specimen processing for steroid testing.

B-081

Determination of Bottled Mass of Angiotensin I in Candidate Standard Reference Material 998a by Amino Acid Analysis

E. L. Kilpatrick. *National Institute of Standards and Technology, Gaithersburg, MD*

Background: The National Institute of Standards and Technology (NIST) has offered a Standard Reference Material (SRM) 998 Angiotensin I (AT-I) for use in the calibration and standardization of renin functional assays. Each vial of this material contained 0.5 mg of AT-I and was reconstituted by adding a desired quantity of solvent. A replacement lot has been procured having an expected mass of 5 mg in each bottle. The purity of the bulk material was assessed prior to packaging and was determined to be 789 mg/g with expanded uncertainty of 58 mg/g (k=2) using amino acid analysis. The current study seeks to determine the average mass and variation of AT-1 as vialed. The true value is critical to determining the reconstitution protocol for this material, whether to add a known amount of solvent or to weigh out milligram quantities for solubilization.

Methods: Six bottles of the material were selected and approximately 1 mL of 0.01 M hydrochloric acid was added and the mass recorded. The samples were diluted approximately 160-fold and two replicates were taken of each diluted sample. The concentration of AT-1 was determined via amino acid analysis (AAA) using double isotope-dilution tandem mass spectrometry after gas phase hydrochloric acid hydrolysis. Samples were spiked with isotopically labeled free amino acids (isoleucine, leucine, phenylalanine, and valine) either before (n=3) or after (n=3) hydrolysis. Calibration was accomplished using unlabeled amino acids also spiked with labeled amino acids, both without acid hydrolysis. The mass in each bottle was calculated using the determined concentration and known solvent addition and then corrected for purity.

Results: Values were assigned using linear regression of an external ratiometric calibration curve for each individual amino acid. The regression coefficient was found to be > 0.99 in all cases with slope and y-intercept values close to unity and zero, respectively. The average mass determined for AT-1 was 5.46 mg (5.0 %cv) and 6.00 mg (4.1 %cv) for the groups for which the internal standard was added either pre-hydrolysis or post-hydrolysis, respectively. The means of the two groups were not shown to be different (t-test, p > 0.05) and the results were combined to yield an average of 5.7 mg (6.6 %cv).

Conclusion: The evaluation of the mass contained within each bottle of candidate SRM998a, Angiotensin-I was performed using amino acid analysis and the variation was determined. The bottle to bottle variation shown may be acceptable for use as a calibrant prepared by fixed volume addition in functional assays but will require investigation into each specific case. The larger mass contained in SRM998a will offer the additional ability to measure out specific quantities of material to avoid the bottle-to-bottle variability.

B-082

High-Throughput LC-MS/MS Measurement of 17-Hydroxyprogesterone in Human Blood Serum for Research Purposes

J. M. Di Bussolo[1], R. Castillo[2], A. Shah[2], H. Othman[2]. [1]*Thermo Fisher Scientific, San Jose, CA,* [2]*BioReference Laboratories, Elmwood Park, NJ*

Background: 17-Hydroxyprogesterone (17-OHP) is a biosynthetic precursor to other steroids such as corticosteroids, androgens, and estrogens. It is converted to 11-deoxycortisol by 21-alpha-hydroxylase or to androstenedione by 17, 20 lyase. Researchers investigating how these enzymes function need to measure 17-OHP within an analytical range of 10 to 1,000 ng/dL (0.3 to 30 nmol/L) in blood serum.

Methods: 17-0HP was measured in blood serum using a multi-channel ultra high-performance liquid chromatography (UHPLC) coupled to a triple-quadrupole mass spectrometer (MS) with atmospheric-pressure chemical ionization (APCI). Sample preparation involved extracting specimens with methyl-t-butyl ether after spiking them with 17-OHP-D_8 internal standard (IS). After evaporating and reconstituting the extracts to concentrate them two-fold, injections were made into a 4-channel UHPLC system. A 4-minute water-to-methanol gradient eluted the analyte and IS through a column, packed with solid-core silica with hydrocarbon groups bonded to its surfaces, into the APCI source. Selective-reaction monitoring (SRM) within a 1-minute data window produced quantitation and conformation chromatographic peaks.

Results: Confirmation/quantitation ion ratios among calibrators, QCs, and specimens were within 20% of averages calculated from the calibrators. Method precision, assessed as percent coefficient of variation (%CV) of peak areas from 20 replicate injections of two pools of test specimens, were better than 5% and 6% for intra- and inter-batches, respectively. Carryover, measured in blanks immediately following injections of the highest calibrator, never exceeded 0.1%. Specimen IS peak areas averaged 87% relative to the averaged IS peak areas in calibrators and QCs, indicating low ion-suppression by matrix. The desired analytical range from 10 to 1,000 ng/dL (0.3 to 30 nmol/L) was achieved and was consistently linear ($r2 \geq 0.999$ with 1/X weighting). For method comparison, 40 donor samples were analyzed and results were compared with those from a reference lab. 17-OHP values among these samples ranged from 13 to 789 ng/dL and the percent difference between two analytical methods did not exceed 20% for 95% of the samples. Sample throughputs were 14, 28, 42 or 56 injections per hour when multi-channeled across 1, 2, 3 or 4 channels, respectively.

Conclusion: We developed a sensitive, robust, high-throughput quantitation assay for 17-hydroxyprogesterone which can measure 10 to 1,000 ng/dL (0.3 to 30 nmol/L) in blood serum. The LC-MS/MS method can be multi-channeled with other APCI methods.

B-083

Simple and Fast Determination of Cyclosporine A in Whole Blood by LC-MS/MS for Therapeutic Drug Monitoring

B. P. Paulo[1], I. Sousa[1], C. F. Guimarães[1], L. R. Silva[2], D. Zauli[1]. [1]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil,* [2]*Hermes Pardini Institute (Toxicology Division), Belo Horizonte, Brazil*

Background: Cyclosporine is a cyclic polypeptide of 11 amino acids produced by a fungal species (*Beaveria nivea*) which has profound immunosuppressive properties, particularly acting as an inhibitor of calcineurin, being responsible for activating an important signal transduction pathway of T cell activation. The result of the inhibition is a decrease in maturation of T lymphocytes and reduction in lymphokine production, including IL-2. Cyclosporine is available in capsules and in solution for oral and intravenous use. Because of its variable absorption, the usual maintenance dose varies greatly and proper dosing requires for monitoring drug levels. Cyclosporine undergoes extensive hepatic metabolism and, because of its interaction with the cytochrome P450 system (CYP3A4), is susceptible to severe drug-drug interactions. **Objective:** The objective of this work was develop and validate a selective and simple analytical method for cyclosporine A determination in whole blood by LCMS/MS. **Methods:** The chromatographic separation was performed on Supelco Ascentis Express C18 column (20 mm X 2.1 mm X 2.7 µm) held at 50 °C using a gradient separation constituted of mobile phase A (5 mM ammonium formate with 0.1% of acid formic solution) and B (methanol with 5 mM ammonium formate and 0.1% of acid formic). The chromatographic run time was 1.7 min. All experiments were performed on Waters XEVO TQD LC-MS/MS system with Acquity UPLC system. The source was operated in positive mode. The sample preparation was carried out adding a stable isotope internal standard (cyclosporine-d4), $ZnSO_4$ solution, and acetonitrile for protein denaturation. The mixture was stirred, centrifuged and 10 µL of the supernatant was injected into

LC-MS/MS system. The method was validated achieving a LoD of 5.2 ng mL^{-1} and a linearity between 25.0-625.0 ng mL^{-1}. A 1:4 dilution was also validated, extending the reportable range until 2,500.0 ng mL^{-1}. The recovery was between 89.6 and 102.2%, and the intra and inter-day precision was less than 7.1%. **Conclusion:** The LC-MS/MS method has been developed and validated successfully. The method is simple, rapid and precise, and can be applied for therapeutic drug monitoring of cyclosporine A in whole blood.

B-084

Development and Validation of Tacrolimus in Whole Blood by LC-MS/MS

N. L. Dias, M. R. Diniz, B. F. P. Paulo, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Tacrolimus is an immunosuppressive agent commonly used to prevent rejection of transplanted organs. Because of the narrow therapeutic range of this drug class, monitoring the levels in blood is part of immunosuppressive therapy after organ transplantation. The liquid chromatography tandem mass spectrometry (LC-MS/MS) method is considered the gold standard in therapeutic monitoring of this drug. **Objective:** To develop and validate a LC-MS/MS analytical method for determination of tacrolimus in whole blood. **Methods:** Sample preparation was performed adding an internal standard solution (ascomycin) to 100 µL of sample, and then $ZnSO_4$ solution. The mixture was stirred and a liquid-liquid extraction using ethyl acetate was performed. The supernatant was dryness, ressuspended with methanol solution and injected into LC-MS/MS system. The chromatographic separation was performed on Supelco Ascentis Express C18 column (20 mm X 2.1 mm X 2.7 µm) held at 50 °C using an isocratic method constituted by (5:95%) mobile phase A (5 mM ammonium formate with 0.1% of acid formic solution) and B (methanol with 5 mM ammonium formate and 0.1% of acid formic). The chromatographic run time obtained was 1.0 min. All experiments were performed on Waters XEVO TQD with Acquity UPLC system and the source was operated in a positive mode. The linear range achieved was between 1.0 and 51.0 ng.mL^{-1}. The limit of detection (LOD) calculated was 0.2 ng.mL^{-1}. The intra and inter-day precision were less than 12% and the medium range of recovery was between 90 and 105%. **Conclusion:** In conclusion, the LC-MS/MS method has been developed and validated successfully and can be applied in clinical routine for therapeutic drug monitoring of tacrolimus.

B-085

Rapid Determination of Pantothenic Acid in Human Serum by LC-MS/MS

N. L. Dias, M. R. Diniz, B. F. P. Paulo, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Pantothenic acid (Vitamin B5) is a precursor of coenzyme A, notable for its role in the metabolism of fatty acids. Its deficiency can cause fatigue, sleep disturbances, impaired coordination, and nausea. **Objective:** to develop and validate a LC-MS/MS method for determination of pantothenic acid levels in serum. **Methods:** Chromatographic separation was performed on an Agilent 1290 Infinity LC system equipped with a Poroshell 120 PFP column (100 mm x 2.1 mm, 2.7 µm) and a mobile phase constituted by water:methanol (70:30, v/v) with 0.1% de formic acid at a flow rate of 0.3 mL min^{-1}. The chromatographic run time obtained was 2.0 min. Detection was made on Agilent 6460C triple quadrupole system with electrospray ionization (ESI+) for quantification of Vitamin B5. The sample preparation was a simple protein precipitation with trichloroacetic acid solution and 25 µL of a labelled internal standard solution (pantothenic acid-$^{13}C_3$,^{15}N). The linear range achieved was between 10.0 and 4,000.0 ng mL^{-1}. The medium range of recovery was between 90 and 100%. The coefficient of variation ranged from 0.4 to 6.0% and the limit of detection calculated was 4.7 ng mL^{-1}. **Conclusion:** In conclusion, the method has been developed and validated successfully, and can be applied in clinical routine for determination of vitamin B5 in serum.

B-086

Rapid Identificationof Nocardia spp.usingMatrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry and Susceptibility Analysis of Nocardia

y. qing, h. chen, l. fang. *The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China*

Nocardia species are ubiquitous environmental bacteria with increasing frequency from clinical specimens, especially those from immunocompromised patients. Identification of the Nocardia species is important because different species vary in their susceptibilities to various antimicrobial agents.The traditional identification of Nocardia strains to the species level is a difficult and time-consuming process.In this study, 75 clinical isolates of Nocardia spp. collected in the lasted 5 years were characterized by the Bruker Biotyper MALDI-TOF system using the BDAL 5989 (v5) Biotyper databases, and gyrB gene sequencing was used as reference methods for species identificaiton. The antimicrobial sensitivity of nocardia was also determined by broth microdilution testing.The gyrB gene sequencing successfully assigned to species level, the most common isolated was *N. farcinica* (n = 26),followed by *N.cyriacigeorgica* (n = 16), *N.brasiliensis* (n=11), *N.nova* (n=7),*N. otitidiscaviarum* (n=6),*N. abscessus* (n = 3) and *N.terpenica*(n=2),*N. asteroides*(n=1), *N.asiatica*(n=1), *N.concava*(n=1), *N. transvalensis*(n=1).Following MALDI-TOF analysis, species level identifications were achieved at a score cutoff of ≥1.5 after direct extraction (62/

75, 82.7%). All isolates were sensitive to Trimethoprim-sulfamethoxazole and linezolid,the sensitivity to amikacin was 97.3%,and sensitivity to other various antimicrobial agents vary in different species.The sensitivity of *N. farcinica* to imipenem,amoxicillin-clavulanic acid,minocycline >90%.The sensitivity of *N.cyriacigeorgica* to imipenem,tobramycin,ceftriaxone >80%. All *N.brasiliensis* isolates were sensitive to tobramycin,ceftriaxone,amoxicillin-clavulanic acid, and *N.nova* isolates were sensitive to imipenem,cefepime, minocycline, clindamycin. Overall, the Bruker Biotyper MALDI-TOF system can provide rapid and accurate identification and is helpful for the rational selection of antimicrobial agents for Nocardia spp.

B-087

Difference of Urine Nicotine, Nicotine Metabolites, and Anabasine between Regular Tobacco Smokers and Heat-Not-Burn Tobacco Smokers

S. Moon[1], J. Lee[1], S. Hwang[1], Y. Lee[1], S. Lee[1], Y. Yi[1], S. Lee[2], E. Park[2], Y. Cho[2], H. Kim[1]. *[1]Pusan National University Hospital, Busan, Korea, Republic of, [2]Pusan National University Yangsan Hospital, Yangsan, Korea, Republic of*

Background: Recently marketed heat-not-burn tobaccos (HnB) electronically heat the tobacco to release aerosolized nicotine, not burn down the leaves. With extensive promotion by major tobacco companies, its market share in Republic Korea is increasing steeply in recent years. Reliable measurement of nicotine metabolites in smoker's urine could be achieved by using LC-MS/MS. The aim of this study is to validate the quantification method of urinary nicotine (NIC), cotinine (COT), trans-3-hydroxycotinine (3HC) and anabasine (ANA). These metabolites were compared between the urine of regular cigarette smokers and HnB smokers.

Methods: Briefly centrifuged urine 200 µL was thoroughly mixed with dichloromethane including isotope labeled compounds. The supernatant was acidified then evaporated. After reconstituted with the mobile phase, samples were injected into Agilent 1290 series (Agilent) connected with API 4000 (Sciex) on positive ion mode. Four analytes were separated by gradient elution on Synergi Polar RP (4 µm, 75×2.0mm). Precision, accuracy, linearity, matrix effect, dilution integrity, and stability were validated. Regular tobacco smokers were recruited by the tobacco abstinence program in the smoking control center. HbN smokers were recruited from family medicine clinics, healthcare workers, and volunteers. **Results:** Measurable range of NIC, COT, 3HC, and ANA in urine was 10~2,500 ng/mL. Precision was within an acceptable range. Matrix effect evaluated with 6 children urines was minimal. Metabolites in urine were stable for 3 days when refrigerated and stable for months when frozen. Dilution with negative urine did not affect performance seriously. For regular tobacco smokers, 70 persons showed 70~5,000 ng/mL of NIC, 100~900 ng/mL of COT, 50~2,000 ng/mL of 3HC, 50~2,000 ng/mL of ANA. For HnB smokers, 20 persons showed relatively lower nicotine metabolites in urine. **Conclusion:**

We developed and validated the method for quantification of urine nicotine metabolites. Little is known about newly introduced HnB tobacco and its effect. Our research is ongoing and gathering more samples from HnB smokers.

B-088

Comparison of an In-house Method and the Commercial MSMS Vitamin D Kit for the Quantitative Determination of 25-Hydroxy-Vitamin D2 and 25-Hydroxy-Vitamin D3 in Human Serum Samples by Liquid Chromatography-Tandem Mass Spectrometry

J. CHOI, S. CHOI, E. LEE, G. OH. *Eone Laboratories, Incheon, Korea, Republic of*

Background: Vitamin D plays a pivotal role to maintain blood calcium and phosphorus levels which is essential for life processes. Vitamin D exists in two forms in human serum, which are vitamin D2 and D3. The 25-hydroxylase enzyme converts Vitamins D2 and D3 to 25-OH Vitamin D in the liver. We have evaluated two methods for quantitation of 25-hydroxy-vitamin D2 and 25-hydroxy-vitamin D3 which are an in-house (IH) method and the MSMS Vitamin D kit (PerkinElmer, Waltham, MA, USA) by LC-MS/MS. **Methods:** The in-house method for quantitation of 25-hydroxy-vitamin D2 and 25-hydroxy-vitamin D3 was developed using liquid chromatography-tandem mass spectrometry and clinically evaluated with a retrospective analysis in human serum samples. The commercial Kit was using MSMS Vitamin D kit (PerkinElmer, Waltham, MA, USA). **Results:** 76 samples were compared. The 25-hydroxy-vitamin D2 result ranges of in house method were from 0.10 to 1.65 ng/mL, and those of commercial kit were from 0.11 to 1.47 ng/mL respectively. And, the 25-hydroxy-vitamin D3 result ranges of in house method were from 9.30 to 86.31 ng/mL, and those of commercial kit were from 11.17 to 119.61 ng/mL respectively. The correlation coefficients (r) between those two methods were 0.9540 in D2 and 0.9421 in D3 respectively. **Conclusion:** Quantitations of 25-hydroxy-vitamin D2 and 25-hydroxy-vitamin D3 are widely used as a means of assessing vitamin D deficiency status because of their clinical significance in a variety of disorders. The commercial kit method is useful in comparison to the in-house method and does not need to manufacture the materials required for procedures. Also, the quality control of the commercial kit is relatively easier than that of the in-house method. And, the peak to detect is clearly expressed due to the addition of methylamine in the commercial kit. However, the IH method is less expensive than the commercial kit.

B-089

Rapid Discrimination of Methicillin-Sensitive and Methicillin-Resistance *Staphylococcus aureus* by MALDI-TOF Mass Spectrometry

X. Liu[1], T. Su[2], L. Jiang[1], Z. Zhao[2]. [1]*Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China,* [2]*Weill Cornell Medicine, New York, NY*

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major clinical pathogen responsible for both hospital- and community-acquired infections worldwide. It causes mild to severe infections, which is sometimes difficult to treat due to its resistance to multiple antibiotics. Therefore, the selection of the right antibiotics at the right time is most important for MRSA infection control. However, conventional antibiotic susceptibility testing in clinical laboratories is still time-consuming, which requires at least 10 hours following bacterial isolation. The delay of right antibiotic treatment contributes to longer hospitalization stay, higher costs, and increasing in-hospital mortality when the initial antibiotic treatment is inappropriate. In recent years, Matrix-assisted laser-desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) has been widely used for bacterial identification in clinical microbiology laboratories, as it is faster, more accurate and cost-ineffective than conventional biochemical tests. Further, explore MALDI-TOF MS in bacterial subspecies discrimination provides a potential to develop a rapid and accurate method for antibiotic susceptibility testing. Therefore, we tend to establish a MALDI-TOF MS-based methodology for MRSA typing with machine learning algorithms.

Methods: A total of 452 clinical *S. aureus* isolates (including 193 MRSA and 258 Methicillin-sensitive *Staphylococcus aureus* (MSSA) strains) were collected from 2012 to 2018 at Clinical Laboratory, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, China. A conventional antibiotic susceptibility testing (VITEK-2 compact, BioMérieux) was utilized to determine MRSA and MSSA of all isolates. All isolates were identified by MALDI-TOF MS (VITEK MS, BioMérieux), and subsequently the mass spectra ranging from 2,000 to 20,000 Da were collected and exported. Raw MS spectra were smoothed, baselines corrected and peaks detected. MS Peaks were aligned by fitting warping function in "MALDIquant". "MALDIquant" and "MALDIquantForeign" R packages were employed to process the MALDI-TOF MS spectra and integrate m/z values from different samples. Selected m/z features and their corresponding intensities were used for binary classification. Radial Basis Function Support Vector Classifier (RBF SVM) was applied to distinguish MRSA from MSSA by using the training set. The performance was validated by the test set.

Results: Total 1344 m/z features were integrated (tolerance=2000 ppm at 4000Da) and 135 m/z values with high probability of occurrence across all the samples were selected as discriminative features. 320 *Staphylococcus aureus* clinical isolates (137 MRSA and 183 MSSA) were used as the training set to establish the predictive model. It shows 96.45% accuracy, 93.28% sensitivity and 98.86% specificity in discrimination of MRSA from MSSA in the training set. While in the testing set of 132 *Staphylococcus aureus* clinical isolates (57 MRSA and 75 MSSA), there is 82.58% accuracy, 77.19% sensitivity, and 86.67% specificity.

Conclusion: A rapid and accurate method for discriminating MRSA from MSSA was established based on MALDI-TOF MS. Considering that this method is easy to implement in routine microbiology laboratories, it suggests a cost effective and time efficient alternative to conventional antibiotic susceptibility testing in the future to improve health care.

B-090

UPLC-MS/MS Analysis of DHT, DHEA, Testosterone, Androstenedione, 17-OHP and Progesterone in Serum for Clinical Research

R. Wardle, D. Foley, L. Calton. *Waters Corporation, Wilmslow, United Kingdom*

Background: Here we evaluate an offline automated method for the measurement of serum dihydrotestesterone (DHT), dehydroepiandrosterone (DHEA), testosterone, androstenedione, 17-hydroxyprogesterone (17-OHP) and progesterone, enabling steroid profiling for the investigation of metabolic dysfunction biomarkers for clinical research. A UPLC-MS/MS method was developed using a mixed-mode Solid Phase Extraction (SPE) sorbent in 96-well plate format, improving workflow and reducing sample preparation time. Chromatographic resolution between structurally related steroid species was achieved to help obtain highly precise and accurate data, particularly at low physiological concentrations. **Methods:** Certified steroid hormone reference material purchased from Sigma Aldrich (Poole, UK) were used to create calibrators and QC materials in 1% Bovine Serum Albumin (BSA) in Phosphate Buffered Saline (PBS). Serum samples purchased from NEQAS (Birmingham, UK) for testosterone, androstenedione and 17-OHP were analyzed and concentrations were compared to the EQA MS mean for each steroid hormone. Lyophilized serum samples purchased from RCPA (NSW, Australia) for DHT were compared to the target values. Additionally, samples previously analysed by an independent LC-MS/MS clinical research method were evaluated for comparison. Sample preparation was automated offline using a Tecan® Freedom Evo 100. 100 µL serum samples were pre-treated with internal standard, methanol and water. Waters Oasis MAX µElution SPE was performed, allowing direct injection of the SPE eluate. Using an ACQUITY UPLC I-Class system, samples were injected onto a 2.1 x 50 mm Waters CORTECS C_{18} column with an in-line 0.2 µm filter using a water/methanol/ammonium fluoride gradient and quantified with a Waters Xevo TQ-S micro mass spectrometer. **Results:** The developed method was shown to be linear over the measuring range for the serum steroid hormones. Coefficients of variation (CV) for total precision and repeatability on five separate days for low, mid and high QC samples were ≤6.5% (n=25) for all analytes. Analytical sensitivity investigations performed over five occasions demonstrated a CV >20% (S:N <10) at 0.025 ng/mL for DHT, 0.1 ng/mL for DHEA, 0.005 ng/mL for testosterone, 0.01 ng/mL for androstenedione, 0.01 ng/mL for 17-OHP and 0.005 ng/mL for progesterone. Matrix Factor experiments demonstrated the internal standard compensated for ion suppression observed in the method, with accuracies of 95-101% and CVs ≤3.4% for the serum steroids. The method has shown to be analytically selective through separation of isobaric steroid species and matrix specific interferences such as albumin, intralipid, cholesterol, triglycerides and bilirubin. Agreement between this analytical method and the EQA LC-MS mean values have been demonstrated with mean method bias of -1.4%, +0.2% and -5.6% for testosterone, androstenedione and 17-OHP, respectively. Agreement between this analytical method and RCPA target values for DHT was +4.9% mean bias and method comparison to an independent LC-MS/MS clinical research method for DHT demonstrated a mean bias of -6.6% for the samples analysed. **Conclusions:** This offline automated clinical research method demonstrates excellent linearity, analytical sensitivity, selectivity, precision and accuracy. The method quantifies serum samples for DHT, DHEA, testosterone, androstenedione, 17-OHP and progesterone using UPLC-MS/MS, providing sample tracking capabilities and high sample throughput capabilities whilst minimizing operator error. For Research Use Only. Not for use in diagnostic procedures.

B-091

Plasma Bradykinin Concentrations in Patients During Septic Shock: A Novel LC-MS/MS Assay

M. Lindström[1], M. Valkonen[2], N. Tohmola[1], R. Renkonen[3], T. Strandin[3], A. Vaheri[3], O. Itkonen[1]. *[1]HUSLAB, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [2]Division of Intensive Care Medicine, Department of Anesthesiology, Intensive Care and Pain Medicine, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [3]Medicum, Faculty of Medicine, University of Helsinki, Helsinki, Finland*

Background: Sepsis is a serious clinical condition, where immediate therapeutical intervention is crucial for patient survival. However, the exact mechanisms sepsis still remain unclear. The kinin system, and especially bradykinin has recently been studied in order to illuminate sepsis progression. Increase of circulating bradykinin is considered a major factor in blood vessel leakage leading to fall in blood pressure. Due to the rapid progression of the condition, human experimentation is difficult. Also, bradykinin concentration in blood is usually very low and the molecule degrades quickly. A need for a sensitive and straightforward bradykinin assay is apparent. This work describes the development and validation of a liquid chromatography tandem mass spectrometric (LC-MS/MS) method for quantification of bradykinin in human plasma.

Methods: Blood samples from laboratory staff (n=10) were drawn into pre-chilled Monovette syringes containing inhibitors for enzymatic degradation [1]. The sample was kept on ice until plasma was separated by centrifugation 2500 g for 10 min at +4 ºC. For preparation of quality assurance samples, plasma was spiked with 2, 10, 50, and 400 nmol/L bradykinin (Innovagen AB). These were also used for intra- and inter-assay reproducibility testing. Sample stability at -20 ºC was studied for 12 months. Calibrators (0.25-500 nmol/L) were prepared in water. We mixed 500 µL of calibrator or plasma sample with 100 µL of stable isotope labeled bradykinin (IS; $^{13}C_9$, ^{15}N -bradykinin, Innovagen AB) solution. Samples were then extracted using Waters Oasis WCX µElution plates. Chromatographic separation was performed on a Phenomenex Biphenyl column using acetonitrile and 90 mmol/L ammonium formate, pH 4 for elution. For MS detection, transitions of m/z 531 → 175.4 (bradykinin) and m/z 535 → 175.1 (IS) were followed. Calibration curve was derived using $1/x^2$ weighted linear least-squares regression (Analyst software Version 1.6.2, AB Sciex).

Results: Intra- and inter-assay reproducibility was 3.2-6.5%. The assay was linear (coefficient of variation and relative error <20%) over a concentration range of 0.25-1000 nmol/L. Sample matrix suppressed bradykinin ionization by 52%, but the internal standard compensated for this. Recovery of added bradykinin (1, 10, 100 and 300 nmol/L) was on average 81% (range 73-84%). Plasma samples spiked with 100 nmol/L bradykinin were stable at -20 °C for 12 months (RE <15%).

Conclusion: An LC-MS/MS assay was successfully developed and validated for quantification of bradykinin in plasma samples. The assay will be useful for research and clinical purposes.

B-092

Measurement of Monoclonal Proteins by QIP-MS Aids in the Interpretation of Equivocal Laboratory Results

B. Pepperkok[1], S. Busch[1], T. Peters-Regehr[2], J. Bartel[1]. *[1]MVZ Labor Dr. Limbach, Heidelberg, Germany, [2]The Binding Site GmbH, Schwetzingen, Germany*

Background: Accurate identification and quantification of clonal immunoglobulins and free light chains (FLC) is an important part of the diagnostic algorithm for monoclonal gammopathies. This can be challenging depending upon the isotype, level of production and laboratory method used. Identification of monoclonal proteins by nephelometric/turbidimetric assays can be further confounded by antigen excess (AgXS) and laboratory methods are routinely employed to manage this phenomenon. Here we use quantitative immunoprecipitation mass spectrometry (QIP-MS) to provide a better understanding of 3 challenging samples.

Methods: Routine evaluation of monoclonal proteins includes serum protein electrophoresis, serum immunofixation (IFE) and serum FLC testing (Freelite, The Binding Site). To avoid the issue of AgXS, FLC testing is performed at 2 dilutions irrespective of the FLC ratio. Three patient samples (2 IgMλ [Patients A & B] and 1 IgAλ [Patient C]) with equivocal IFE results and apparent FLC AgXS were assessed using isotype specific paramagnetic beads (IgG, IgA, IgM, κ, λ, free κ and free λ). Light chain mass spectra were acquired using a Bruker microflex LT Smart MALDI-TOF mass spectrometer.

Results: Turbidimetric FLC analysis indicated AgXS, however this was not supported by reaction kinetics for patients A or B. In all cases QIP-MS confirmed the presence of the gross intact monoclonal protein detected by IFE (Patient A IgMλ MW 22540Da; Patient B IgMλ MW 22714Da; Patient C IgAλ MW 22656Da). Moreover, additional intact monoclonal proteins were identified which were below the sensitivity of IFE (Patient A IgMλ MW 22682Da; Patient B IgMλ 22858Da; Patient C 2 x IgMλ MW 22708Da and 22536Da). The quantity of FLC by QIP-MS refuted the AgXS findings in patients A and B with only small clones present above the polyclonal background. In contrast for patient C there was clear evidence of FLC production.

Conclusion: QIP-MS was in agreement with IFE for the detection of major clones and also identified additional monoclonal proteins, providing novel insight into tumour dynamics. The pentameric structure of IgM presents a challenge for analysis by immunoassay techniques. By overcoming such challenges, QIP-MS may prove an invaluable tool for interpretation of equivocal results from routine tests.

B-093

Determination of Sirolimus in Whole Blood by LC-MS/MS for Therapeutic Drug Monitoring

B. F. P. Paulo[1], C. F. Guimarães[1], L. R. Silva[2], N. L. Dias[1], I. M. A. Souza[1], M. R. Diniz[1], D. Zauli[1]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Toxicology Division), Belo Horizonte, Brazil*

Background: Sirolimus is a macrocyclic lactone produced by *Streptomyces hygroscopicus*, which selectively blocks the transcriptional activation of cytokines, inhibiting its production. It is indicated for the prophylaxis of heart, liver, lung, and renal transplant rejection. Monitoring of Sirolimus concentrations is recommended for all patients, especially in those with altered drug metabolism, in patients ≥ 13 years weighing less than 40 kg, in patients with hepatic impairment, and during concurrent administration of strong CYP3A4 inducers and inhibitors. **Objective:** Develop and validate a selective and simple analytical method for Sirolimus determination in whole blood by LCMS/MS. **Methods:** The sample preparation was carried out adding a stable isotope internal standard (sirolimus-d3), $ZnSO_4$ solution, and acetonitrile for protein denaturation. The mixture was stirred, centrifuged and injected into LC-MS/MS system. The chromatographic separation was performed on Supelco Ascentis Express C18 column (20 mm X 2.1 mm X 2.7 µm) held at 50 °C using a gradient separation constituted of mobile phase A (5 mM ammonium formate with 0.1% of acid formic solution) and B (methanol with 5 mM ammonium formate and 0.1% of acid formic). The chromatographic run time was 1.2 min. All experiments were performed on Waters XEVO TQD LC-MS/MS system with Acquity UPLC system and the source was operated in positive mode. **Results:** The method was successfully validated achieving a linearity between 2.0-52.0 ng mL^{-1} and LoD of 0.2 ng mL^{-1}. The intra and inter-day precision was less than 7.1%, and the recovery was between 96.1 and 110.4%. Three CAP interlaboratory samples were tested and a good concordance was obtained with the overall results: standard deviation index of 1.9; 0.0; -0.2 for low, medium and high values, respectively. **Conclusion:** The LC-MS/MS method for determination of Sirolimus in whole blood has been developed and validated. The method is simple, fast, precise, and can be used for therapeutic monitoring in transplanted patients.

B-094

Analysis of Fat Soluble Vitamins in Serum using UPLC-MS/MS for Clinical Research

L. J. Calton, M. Wills, G. Hammond. *Waters Corporation, Wilmslow, United Kingdom*

Background: Extraction and analysis of vitamin A and E by LC-MS/MS has historically involved laborious sample preparation and lengthy analysis time with high solvent consumption, whilst vitamin K_1 is a challenging analyte due to its hydrophobicity (logP 9.70). Phospholipids, which are present in high levels in serum samples, elute in the same chromatographic region as vitamin K_1, causing ion suppression when performing reversed-phase UPLC-MS/MS. Clinical research methods have been developed using a reverse-phase Solid Phase Extraction (SPE) sorbent in 96-well plate format that minimizes interference from phospholipids. This sample preparation research method in combination with an analysis time of only 3 minutes, reduces solvent consumption and allows for high throughput sample analysis.

Methods: Certified reference materials were used to create in-house calibrators and QC materials in stripped serum. Samples were pre-treated with internal standards, centrifuged, and for vitamin A and E analysis a fixed volume of supernatant was di-

luted prior to SPE using Waters™ Oasis™ PRiME HLB µElution plates. All samples were quantified using a Waters ACQUITY UPLC™ I-Class FTN/Xevo™ TQD System with a 2.1x50 mm HSS PFP column and a water/methanol/ammonium acetate/formic acid gradient.

Results: For vitamin A and E the method was shown to be linear over the measuring ranges (100-2000 ng/mL) and (1.1-21.1 µg/mL) respectively. Coefficients of variation (CV) for total precision and repeatability of low, mid and high QC samples (n=25) were all ≤6.9%. No significant carryover was observed from high concentrations serum samples into serum blanks and over-range samples were successfully diluted (1:4) with accuracies ranging from 86% to 102%. Analytical sensitivity (bias ≤15% and CV ≤20%, over 5 occasions) was shown to be 50 ng/mL for vitamin A and less than 1.1 µg/mL for vitamin E. EQA samples demonstrated good agreement between this analytical method and the EQA ALTM mean values, with mean bias of -7.0% for vitamin A and -10.9% for vitamin E. For vitamin K_1 the method was shown to be linear over the range 0.1 - 20 ng/mL, with precision performance ≤10%. Structurally related compounds were chromatographically separated, and endogenous interference studies gave recoveries within 85-115% for all vitamins.

Conclusion: We have successfully quantified vitamin A, vitamin E and vitamin K_1 in serum using SPE with UPLC-MS/MS for clinical research purposes. This method demonstrates good linearity, precision and accuracy, whilst removing phospholipids and providing high throughput capabilities. For Research Use Only. Not for use in diagnostic procedures.

B-095

Development of a Fully Integrated Automated Solution for QIP-MS Sample Preparation

S. Brusseau[1], M. Rizzioli[1], N. J. Wright[1], L. Ouverson[2], G. Harbach[3], S. J. Harding[1], G. L. F. Wallis[1], J. A. Ashby[1], S. J. North[1]. *[1]The Binding Site, Birmingham, United Kingdom, [2]The Binding Site, Rochester, MN, [3]Tecan Trading AG, Männedorf, Switzerland*

Background: The development of a mass spectrometry approach to the identification of polyclonal and monoclonal immunoglobulins published by Mills *et al*., represented an exciting development in the field of monoclonal gammopathy patient management. However, the challenges of converting this pioneering research to an IVD compliant assay/device are not inconsiderable. Here, for the first time, we present quality control data for the reproducible production of paramagnetic beads aimed at identifying IgG, IgA, IgM, kappa and lambda isotypes. Furthermore, all data were obtained using a single integrated liquid handler able to perform parallel processing of primary samples and output fully prepared MALDI target plates ready for mass spectrometric analysis. Moreover, this was achieved in a clinically relevant timescale and resulted in robust, high precision performance, which bodes well for placing this technology into routine laboratory use.

Methods: Monospecific bead reagents were manufactured by covalent attachment of modified sheep polyclonal antibodies (anti-IgG, -IgA, -IgM, kappa and lambda) to blocked paramagnetic microparticles. All stages of pre-analytical sample processing, from bead dispense, analyte capture, light chain release and MALDI target plate preparation, were performed on a custom-designed Tecan Fluent® system using a fully automated workflow. Light chain spectra were acquired by MALDI-TOF mass spectrometry and used as a performance indicator for batch consistency, reagent stability and system reproducibility. Preliminary clinical performance of the fully integrated system was investigated using a cohort of IFE positive patient samples.

Results: There was no significant difference in light chain capture from reference serum (1-way ANOVA, 95% confidence) between independent batches of equivalent specificity. Batch-to-batch consistency was further confirmed by the κ/λ peak intensity ratio for the anti-G (1.82, CV=1.89%), anti-A (0.96, CV=4.65%) and anti-M (1.35, CV=4.35%) reagents. Integrity of the reagents was demonstrated by the absence of reagent-derived sheep light chain in the mass spectra, and analyte binding activity remained stable after 100 days storage at 22°C (equivalent to 12 months at 4°C). In reproducibility tests, comparable light chain signals were observed in 1184 replicate spots with a spotting hit-rate of 100%. Spotting precision (CV=4.43%) was established using a mixture of IgG kappa and IgG lambda proteins replicated over 480 spots. Following parallel processing of 76 IFE-positive patient samples with all five reagent specificities, the fully automated QIP-MS system identified the unequivocal presence of a monoclonal protein in all cases (13 x IgGκ, 14 x IgGλ, 16 x IgAκ, 13 x IgAλ, 14 x IgMκ, 6 x IgMλ).

Conclusion: Standardisation of reagent manufacture and the development of a single pre-analytical handling solution with reliable spotting and remarkable spot to spot CVs suggest this approach is suitable for routine clinical laboratory use.

B-096

Use of Quantitative Immunoprecipitation Mass Spectrometry to Resolve the Complexity of Plasma Cell Populations in Multiple Myeloma

B. Howard, A. Kuh, L. A. Rezavi, P. Caturegli. *Johns Hopkins Hospital, Baltimore, MD*

Background: The clonal heterogeneity and evolution of neoplastic plasma cells has been demonstrated in patients with multiple myeloma (MM) using double immunofluorescence staining and next-generation sequencing. Myeloma cells can produce complete immunoglobulins with or without excess free light chains (FLC), FLC only or, rarely, no detectable immunoglobulins. These immunoglobulin species are characterized by serum immunofixation electrophoresis (IFE) and turbidimetric quantification of FLC (Freelite assay), two methods that are not always in concordance. Indeed, the majority (~95%) of MM patients have an abnormal FLC ratio but do not always show the abnormal FLC by IFE. Since a light chain secreted by the same plasma cell-clone is predicted to have the same molecular mass, independently of whether it is free or bound to the heavy chain, determination of the molecular mass could be of clinical value. We evaluated the utility of quantitative immunoprecipitation mass spectrometry (QIP-MS) to aid interpretation of four patient-samples with discrepant electrophoretic and turbidimetric results.

Methods: We studied 4 MM patients with gammopathy (2IgGκ, 1IgAλ, 1IgGλ) and unbalanced light chain synthesis, defined as the presence of a complete immunoglobulin but no monoclonal FLC on IFE and abnormal FLC results by turbidimetry. κ and λ serum FLCs were measured using Freelite® on an Optilite® instrument (The Binding Site Group Ltd., UK). For QIP-MS studies, samples were subjected to substrate-specific immunoprecipitation using paramagnetic beads coated with polyclonal anti-IgG, IgA, IgM, total κ, total λ, free κ and free λ. After separation of the light from the heavy chain, light chain mass spectra were acquired using a MALDI-TOF mass spectrometer.

Results: Serum κFLC levels were elevated in the two IgGκ patients (450, 281mg/L), as were the λFLC levels in the IgAλ and IgGλ patients (1152, 827mg/L, respectively), yielding abnormal κ/λ FLC ratios. In the 2 IgGκ patients QIP-MS confirmed the presence of monoclonal IgGκ, with light-chain molecular weights (MW) of 23578 and 23252 Daltons (Da). It also identified smaller κFLC peaks with similar masses, indicating that the light chain arose from the same plasma cell as the heavy chain. In the 23758Da IgGκ patient, QIP-MS detected an additional clone producing IgGκ (MW=23454Da), denoting the presence of multiple clones that were not identified by IFE. QIP-MS identified the IgAλ monoclonal protein (MW=22742Da) with λFLC protein at the same MW; and additional discrete IgGλ (MW=22772Da) and IgMλ (MW=22860Da) subtler clones in the same patient which appeared not to have λFLC production. Finally, in the IgGλ patient there seemed to be normal polyclonal IgG at the predicted light chain MW. However, a large shifted protein was clearly identified as IgGλ by QIP-MS, and there were multiple putatively glycosylated proteins with multiple and sometimes discrete putatively glycosylated λFLCs also present.

Conclusion: The low sensitivity of electrophoresis-based techniques can lead to the erroneous conclusion that FLC or other subtle monoclonal clones are not present. QIP-MS improves the characterization of these patients by showing intact and FLC monoclonal immunoglobulins as well as additional monoclonal peaks, overall supporting previous work for the presence of multiple clones within individual patients.

B-097

Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS): Establishing Reliability and Traceability to the International Serum Standard DA470k

D. Sakrikar[1], J. A. Ashby[2], S. J. North[2], L. J. Ouverson[1], G. L. F. Wallis[2], B. K. Du Chateau[1], S. J. Harding[2], D. R. Barnidge[1]. *[1]The Binding Site, Rochester, MN, [2]The Binding Site, Birmingham, United Kingdom*

Background: Assays developed for routine use in clinical laboratories include controls that are traceable to a well-established standard (e.g. ERM-DA470k pooled serum for routine immunoglobulin measurement). Quantitative Immunoprecipitation Mass Spectrometry (QIP-MS) represents a change in the methodology for the identification and ultimately quantification of polyclonal and monoclonal immunoglobulins. Here, to ensure robust and reproducible performance, we describe the inclusion of an ionization control to confirm MALDI spot crystallization and establish traceability to DA470k using immunoglobulin kappa/lambda ratios measured by immunoassay and confirmed using LC-MS.

Methods: QIP-MS was performed on ERM-DA470k and normal pooled serum UNP001 using modified sheep polyclonal antibodies (anti-IgG, -IgA, and -IgM) covalently attached to blocked paramagnetic microparticles. Sera were incubated with particles, washed, and bound Ig's were eluted and reduced to generate immunoglobulin heavy and light chains. Ig elution buffer also contained a low molecular weight protein serving as an ionization control to ensure the viability of the MALDI matrix spot. A Shimadzu MALDI-TOF MS and a SCIEX LC-MS system were used to generate mass spectra to determine the area under the curve (AUC) for polyclonal light chain molecular mass distributions. For each immunoglobulin isotype, the light chain ratios were determined using Hevylite reagents run on an OptiLite analyzer.

Results: Elutes from three separate IPs (anti-IgG, -IgA, and IgM) were analyzed by MALDI-MS followed by LC-MS. Kappa and Lambda light chain molecular mass distribution were observed in all mass spectra from each heavy chain isotype. AUC ratios for ERM-DA470k ratios were as follows for MALDI-MS and LC-MS: GK/GL = 2.01 & 1.58, AK/AL = 1.18 & 0.91, MK/ML = 1.64 & 1.20. For UNP001, AUC ratios were as follows for MALDI and LC-MS: GK/GL = 1.77 & 1.52, AK/AL = 1.40 & 0.91, MK/ML = 1.73 & 1.11. These values were compared to GK/GL (1.99 & 2.05), AK/AL (1.33 & 1.27), and MK/ML (1.82 & 1.60; ERM-DA470k & UNP001 respectively) ratios observed using Hevylite; an FDA cleared immunoturbidometric assay. Kappa/Lambda ratios observed using MALDI agree with Hevylite ratios within an acceptable limit of ± 15%.

The absence of patient-specific spectral signal due to multiple myeloma associated light chain isotype suppression, or hypogammaglobulinemia in a QIP-MS spectrum may introduce uncertainty in the results. Here, we demonstrate in the presence of 10 mg/L purified polyclonal IgG (simulating hypogammaglobulinemia) an ionization control can confirm MALDI spot viability. Presence of an ionization control in this assay could also detect the accidental presence of agents that could disrupt MALDI spots and interfere with ionization.

Conclusions: The results demonstrate that ERM-DA470k can be used to evaluate UNP001 as a master calibrator for QIP-MS. The findings also show the commutability of the calibrator material for the determination of GK/GL, AK/AL, and MK/ML ratios using orthogonal analytical techniques. Furthermore, the addition of an ionization control is an excellent determinant of the viability of the MALDI matrix spot in QIP-MS assay.

B-098

A Rapid and Sensitive LC-MS/MS Method for Quantitative Analysis of GSK-3 Inhibitors in Mouse Plasma

R. Wei[1], A. Zhou[1], D. Wald[2]. [1]*Cleveland State University, Cleveland, OH,* [2]*Case Western Reserve University, Cleveland, OH*

Introduction:

Glycogen Synthase Kinase-3 (GSK-3) is a dynamic, omnipresent serine/threonine protein kinase that affects multiple signalling pathways important for cellular self-renewal, growth, and survival. Inhibition of GSK-3 displays clinical effectiveness for a variety of diseases such as Alzheimer's disease, type 2 diabetes, mood disorders and cancers. GSK-3 40 and 41 are novel potent analogs of GSK-3 inhibitors that show high specificity and bioavailabilities. A sensitive, robust yet simple analytical method needs to be developed for therapeutical drug monitoring.

Method:

GSK-3 inhibitors were administered to C57BL/6 mice via intraperitoneal injections. Mouse plasma was collected at a consecutive time points. Extraction of the samples from the plasma was carried out using a liquid-liquid extraction method. Samples were analyzed using a Shimadzu Nexera UHPLC system coupled with an AB Sciex Qtrap 5500 mass spectrometer which was operated in the positive ESI mode. The chromatographic separation was achieved on a Waters Atlantis T3 C18 column. MRM mode was used for quantitation of GSK-3 inhibitors in biological samples. The LC-MS/MS system was controlled by Sciex Analyst® software (version 1.6.3)

Preliminary Result and Conclusion:

The structural analog GSK-3 inhibitor 10 was used as the internal standard. The total run time was 5.00 min with retention times of 2.21 min, 2.34 min and 2.58 min for the IS, GSK-3 40 and 41 respectively. The method was validated according to the US-FDA guidance for bioanalytical method validation using CD1 mouse plasma from Innovative Research, Inc. A linear response function was established in the concentration range of 0.5-100 ng/ml with a correlation coefficient > 0.99. The intra- and inter-assay accuracy and precision were ≤ ±4% and ≤ 14% for GSK-3 40 and ≤ ±10% and ≤ 11% for GSK-3 41.The developed method was successfully applied to the measurement of the GSK-3 inhibitors in mouse plasma. This is the first LC/MS method to quantify two novel GSK-3 inhibitors simultaneously.

B-099

Acute Kidney Injury Marker Identification by a Liquid Chromatography-Mass Spectrometry (LCMS)-Based Discovery Approach in Canine Serum Samples

M. Yerramilli, M. Yerramilli. *IDEXX Laboratories, Inc., Westbrook, ME*

Background: Kidney injury is an important cause of morbidity and mortality in canines that may initially be overlooked until symptoms are clinically apparent. While acute kidney injury has historically been classified by severity based on increasing blood creatinine level, there is increasing recognition that early injury-related changes in glomerular filtration rate correlate poorly with blood creatinine levels. For the achievement of best outcomes following injury, more sensitive and specific markers than creatinine are needed to detect early kidney injury and to distinguish which patients are at highest risk due to active progression of injury.

Methods: To identify candidate biomarkers for detection of active kidney injury, we employed a liquid chromatography-mass spectrometry (LCMS)-based discovery approach to compare protein expression profiles in retained canine serum from well-characterized cohorts of canines with kidney disease (creatinine >3 mg/dL) and healthy controls (creatinine <1 mg/dL) from our internal reference laboratory. Specific bioinformatics applications utilized for this approach included the Canis lupus familiaris reference proteome from the UniProt Consortium, and SIEVE 1.0 and Proteome Discoverer 1.1 from Thermo Fisher Scientific.

Results: This approach demonstrated that decreased expression of inosine and increased expression of cystatin B and clusterin were associated with serum from canines with kidney disease in comparison to healthy controls. Targeted review of canines within the kidney disease cohort with serum creatine levels > 8mg/dL confirmed that these differential protein expression trends were present in individual canines with advanced disease. Validation of these markers was completed per Food and Drug Association guidelines.

Conclusion: This LCMS-based approach was instrumental for discovery and quantitation of novel kidney injury biomarkers.

B-100

Quantitative Amino Acid Analysis by LC-MS/MS using a Low-Cost Derivatization Approach and Automated Liquid Handler

W. S. Phipps[1], E. Crossley[2], R. Boriack[2], P. Jones[2], K. Patel[2]. [1]*UT Southwestern, Dallas, TX,* [2]*Children's Medical Center, Dallas, TX*

Background & Aim: Quantitative amino acid analysis (AAA) is central to the diagnosis and management of inborn errors of metabolism. "Gold-standard" ion exchange chromatography (IEX) requires several hours of chromatography per sample and is subject to interferences. To overcome these weaknesses, we developed and validated a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to quantify a total of 28 AAs across 3 matrices (plasma, urine, and CSF). AA derivatization using a repurposed PCR liquid handler was implemented for greater speed and consistency of sample preparation.

Methods: Sample preparation consisted of internal standard addition, deproteinization via sulfosalicylic acid, and placement of deproteinized specimens in a QIAGEN QIAgility™ liquid handler for AA derivatization using aTRAQ™ reagents. Substantial cost reduction was achieved by using half manufacturer-recommended aTRAQ reagent volumes and smaller sample. The LCMS utilized an Agilent UPLC system and SCIEX triple quadrupole mass spectrometer operated in selective reaction monitoring (SRM) mode. Quantification involved single-point external calibration and internal standard normalization against stable-isotope labeled AA analogs. Method validation consisted of determination of analytical measurement range (AMR), interval of linearity, carryover, correlation with in-house methods (ion-exchange chromatography and a UPLC-based method) using patient samples, accuracy in spiked plasma, precision studies, and estimation of matrix effects/ion suppression.

Results: Semi-automated sample preparation required approximately 45 minutes of hands-on time for batch processing of 12 patient samples, compared to up to 2 hours using completely manual procedures. Chromatography separation for in-house AAA was reduced from 3 hours (using IEX) to about 11 minutes per sample. All AA demonstrated linearity over 5 to 2000 µM, with R^2 >= 0.99 (2.5 to 1000 µM for Cys). LLOQ was 5 µM and ULOQ was 2000 µM for most targets. During method correlation, Pearson R > 0.95 was determined for 12 of 20 targets (Ala, Cit, Ile, Leu, Lys, Met, Phe, Tau, Thr, Tyr, and Val) when comparing against both of two current in-house methods (IEX and UPLC-UV), and against one of the currently used methods for the remaining targets. 149 of 153 (97%) AA results in spiked plasma were within ±20% of expected concentration. All measured coefficients of variation (%CV) for derivatized AA were

less than 10% for control samples, with several AA subject to slightly higher %CV in patient plasma. Validated AA included Ala, Arg, ASA, Asn, Asp, Cit, Cys, Glu, Gln, Gly, homocystine, homocitrulline, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Tau, Thr, Trp, Tyr, Val, underivatized Ile, and underivatized *allo*-Ile in plasma, Arg, Lys, and Orn in urine, and Gly/ser in CSF. Detection/quantification of phosphoethanolamine was achieved with final validation pending.

Conclusions: The described quantitative AAA method LCMS enabled substantially faster processing of clinical samples compared to current in-house methods with near cost equivalence, greater specificity, and otherwise similar performance characteristics. This is the first demonstration to our knowledge of AAA involving aTRAQ derivatization at half volumes. The LCMS method is free from optical interferences plaguing traditional chromatography methods and sample preparation is streamlined via a repurposed PCR liquid handler.

B-101

Evaluation between Two LC-MS/MS Protocols and Dimension CSA Immunoassay Method for Determination of Cyclosporine A in Whole Blood

B. F. P. Paulo, C. F. Guimarães, D. Zauli. *Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil*

Background: Cyclosporine A is a potent immunosuppressive agent used by recipients of solid-organ transplants. There are several methods available for Cyclosporine quantification, and the agreement of them is very important for monitoring dosage of this drug. Although LC-MS/MS is the gold standard for therapeutic monitoring of this class of drugs, differences also can be observed in LC-MS/MS protocols. **Objective:** to demonstrate the observed results between two in-house LC-MS/MS protocols and a Dimension CSA immunoassay method for whole blood Cyclosporine dosage. **Methods:** Fifty-eight patient samples (concentration range between 29.0 - 636.0 ng/mL) in immunosuppressant therapy with Cyclosporine A were tested to compare the three methods. The data were evaluated using Passing-Bablok regression and Bland-Altman plot. The first method was a Dimension Systems CSA Assay from Siemens Healthcare Diagnostics, and the others were in-house LC-MS/MS methods with different sample preparation protocols: a protein precipitation (PP) with zinc sulfate and acetonitrile; and a protein precipitation with zinc sulfate solution and then a liquid-liquid extraction with ethyl acetate (LLE). For in-house LC-MS/MS methods, the chromatographic separation was performed on Supelco Ascentis Express C18 column (20 mm X 2.1 mm X 2.7 µm) using a gradient separation constituted of mobile phase A (5 mM ammonium formate with 0.1% of acid formic solution) and B (methanol with 5 mM ammonium formate and 0.1% of acid formic). All experiments were performed on Waters XEVO TQD LC-MS/MS system with Acquity UPLC system. The analysis of slopes of Passing-Bablok regression suggests that there was no statistical difference between PP-Dimension methods [regression equation: 1.043 (±0.051) x + 25.749 (±6.893)], although there was a difference between LLE-Dimension methods [regression equation: 1.071 (±0.034) x + 20.749 (±4.753)]. The Dimension results presents higher values than LC-MS/MS methods, as can be observed by the intercepts, which are different from zero. These values are related to the low selectivity of the immunoassay methods. The difference of results between LLE-PP [regression equation: 1.035 (±0.028) x - 4.506 (±4.748)] is small, but there is at least a proportional difference between both methods. **Conclusion:** According with Passing-Bablok regression, Dimension CSA immunoassay method versus PP LC-MS/MS protocol are comparable, but present a systematic difference on results of Cyclosporine A. The LLE-PP comparison suggest that the choice of the extraction method is very important for Cyclosporine A determination by LC-MS/MS.

B-102

Development of an LC-MSMS-Based Screening Assay for Diagnosis of Cystinuria

P. Quint, A. Lueke, J. Lieske. *Mayo Clinic, Rochester, MN*

Background: Cystinuria is the most common monogenic form of kidney stone disease accounting for up to 1% of adult stones and 8% in children. Normally, nearly 100% of filtered cystine is reabsorbed so that cystine excretion in the urine is very low. Individuals with cystinuria carry an autosomal recessive defect in one of two genes that encode the 2 subunits of proximal tubular transporter required for proper reabsorption of cystine, ornithine, lysine and arginine. Since cystine is poorly soluble in urine, affected individuals are susceptible to recurrent cystine stones. Since cystine stones are not distinguishable from calcium stones on imaging, diagnosis requires capturing a stone for analysis or quantitative measurement of urinary cystine concentration. Many labs perform colorimetric cystine assays that measure the reduced cysteine monomers. These assays are subject to interferences, including from creatinine, and cannot distinguish intact cystine from soluble drug-cysteine complexes when patients are placed upon thiol drugs as a treatment (e.g., tiopronin or d-penicillamine). Thus, we sought to develop an LC-MS/MS assay to quantitatively measure urinary cystine, ornithine, cysteine-drug complexes, and creatinine that could be used to diagnosis and monitor patients on or off therapy. Being able to measure drug effect in urine is important since these agents can have side effects that appear dose related, including rash, allergy, hematologic and liver function abnormalities, and rarely nephrotic syndrome; thus using the lowest effective dose is mandatory.

Methods: Urine is diluted in 0.6 N HCl, combined with internal standards in a 2.0 ml glass autosampler vial, and injected (5 µl) onto a 50 x 3 mm Imtakt Intrada Amino Acid column. Four analytes (cystine, creatinine, ornithine and the tiopronin-cysteine conjugate) are chromatographically separated over 6 minutes using an ACN/H_2O gradient with ammonium formate as a modifier (normal phase). Analytes are monitored in positive-ion mode using the following transitions: Creatinine = 114-72 and 86, Cystine = 241-152 and 74, Conjugate = 282.8-122.0 and 194.2 and Ornithine = 133.1-70.2 and 116.2.

Results: Our results demonstrate good linearity between 0.2 - 15 mg/dL for cystine (R^2 = 0.998), ornithine (R^2 = 0.998) and the drug-cysteine conjugate (R^2 = 0.992); creatinine requires a 1/x curve from 7.5-500 mg/dL (R^2 = 0.998). Creatinine results compared well with those obtained from the Roche enzymatic creatinase assay performed on a cobas c501 with an overall R^2 = 0.96. When spiked into native urine, cystine, the conjugate and ornithine all demonstrated good recovery (cystine mean = 102% range 91-109%; conjugate mean = 101% range 92-113%; ornithine mean = 93% range 81-104%).

Conclusion: We have developed a sensitive, precise, and rapid LC-MS/MS assay to measure urinary cystine, creatinine, ornithine and tiopronin-cysteine conjugate. This test appears suitable as a screening tool for cystinuria, and to monitor patients on treatments including tiopronin.

B-103

Hunting Down the “Ghost”: A Practical Lesson Learned from Investigating an Interference Peak in 1,25-dihydroxy Vitamin D Assay

R. Huang, X. Yi. *Houston Methodist Hospital, Houston, TX*

Background: Troubleshooting of a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay can be challenging in clinical laboratories. Due to the complexity of the assay, a subtle change of any reagent, solvent and instrument used in an LC-MS/MS test could result to a systematic problem affecting all patient testing. In problem troubleshooting, analysis of chromatogram and reviewing testing records could generally help to narrow down the possible causes. We recently took a systematic approach to investigate a “ghost” peak observed in our 1, 25-dihydroxyvitamin D (DHVD) LC-MS/MS assay in patient samples. The DHVD test is used to assess the status of the active form of vitamin D in patients with chronic renal disease or for differential diagnosis of hypercalcemia. **Methods:** Our DHVD assay included an extraction step using AB Sciex Amplifex™ C1000 and S500 cartridges for purification and separation of DHVD from the serum sample. Deuterated internal standards (d6-DHVD2 and d6-DHVD3) were added to the serum sample prior to the extraction. The extracted DHVD and the internal standards were then subject to 4-phenyl-1,2,4-triazoline-3,5 dione(PTAD) derivatization, prior to the LC separation and MS/MS measurements. Without any known instrument problem, an interfering peak was noticed co-eluting with DHVD3 in most patient samples causing positive bias. Systematic troubleshooting approaches were taken to analyze and identify the source of the interfering peak: 1) LC-MS/MS system (e.g. ionization source, mass accuracy, and carryover/or contamination in LC system, mobile phase and column); 2) sample preparation (e.g. extraction solvents and cartridges, glass tubes and sample collection tubes, derivatization reagent, and other consumables). **Results:** No interfering peak was observed in any other analytes (d6-DHVD3, DHVD2 and D6-DHVD2), or in any calibrators or quality control (QC) samples, which were made from stripped blank serum. Therefore, the cause was unlikely to be from LC-MS/MS, but rather from sample preparation. No changes has been occurred in sample collection or patient population and sample source contamination was also excluded by sharing specimens with a reference lab. Not until all other possibilities were excluded, we identified a new lot of extraction cartridges as the source and the “ghost” peak was no longer observed after switching to same cartridges manufactured from a different vendor. Although new lot of calibrators, QCs and columns were required to be validated before use, the new lot of cartridges, solvents, and derivatization reagent were not tracked in our routine procedures. Implementing a new procedure to include a log-sheet keeping track of all involving components in the assay could facilitate the troubleshooting process. **Con-**

clusion: Interfering chromatography issues are commonly seen in LC-MS/MS assays, and depending on the complexity of the methods, troubleshooting could be especially challenging, time-consuming and costly. The troubleshooting processes of LC-MS/MS assays are usually heavily laid on individual laboratories, as manufactures may be of limited help. In this study, we have successfully identified the source of an interfering peak for the DHVD assay, and improved our routine procedures to better track all of our LC-MS/MS assays, which could facilitate troubleshooting process if problem occurs.

B-104

Development of LC-MS/MS-Based Quantitative Assay for the Determination of Plasma Iohexol Clearance in the Assessment of Glomerular Filtration Rate

K. Fankhauser, V. K. Ariyamuthu, B. Tanriover, I. A. Hashim. *University of Texas Southwestern Medical Center, Dallas, TX*

Background: Accurate and reliable assessment of glomerular filtration rate (GFR) is important among others, in the assessment of a donor kidney function, in patients with chronic kidney disease and in patients with cardiovascular disorders, as well as patients on medications known to either affect the kidney (nephrotoxic) or their clearance and metabolism is influenced by glomerular filtration rate. Iohexol, a non-ionic contrast medium, clearance rate is increasingly being used to measure GFR. We describe the development of an LCMSMS-based assay for measurement of Iohexol levels in plasma.

Methods: An UPLC/MS/MS instrument (Acquity TQD, Waters, MA) equipped with BEH C18 (1.7 micm, 2.1 x 100mm) column was used. Mobile phases were formic acid in water (0.1%v/v) and formic acid in acetonitrile (0.1% v/v). A continuous reaction monitoring in a positive mode setting resulted in acceptable ions fragmentations at 821.98 (parent), at 803.80 (quantifier), and at 375.04 (qualifier). Lower limit of detection was determined following serial dilutions of Iohexol standard, whereas analytical measurement range determined using plasma samples spiked with known Iohexol levels. Sample stability was assessed for storage at room temperature, refrigerated at 4C and frozen at -20C. Interference studies were performed for hemolysis index from 74 up to 764, lipemia index 78 to 101, vancomycin levels from 22 to 60 micg/mL, tacrolimus levels from 5.4 to 40.9 ng/mL, cyclosporine levels from 37 to 330 ng/mL, and sirolimus levels from 4.4 to 10.7 ng/mL. Plasma samples (lithium heparin) collected at 0, 10, 20, 30, 50, 80, 120, 180 and at 240 minutes from normal volunteers (n=6) following injection of 3000 mg Iohexol were analysed for Iohexol levels and for Iohexol clearance rates.

Results: Assay development validation exhibited a detection limit of 5 micg/mL, and a linear range up to 1000 micg/mL. Intra- and inter-assay variations were less than 2.7, 5.1, 5.6 at 10, 240 and 592 micg/mL Iohexol respectively. Samples were stable for 24 hours at room temperature, up to 5 days at 4C and up to 14 days when stored frozen at -20C. There was no significant interference from immunosuppressant drugs (selected for their possible co-administration) nor from hemolysis or lipemia. No ion suppression was observed. Blood samples from normal volunteers showed mean Iohexol levels of 549.5, 407.7, 345.1, 278.7, 185.7, 134.8, 82.9, and 61.1 micg/mL at 10, 20, 30,50,80,120,180, and 240, minutes following administration respectively. A dynamic Iohexol clearance report is generated jointly by Clinical Chemistry laboratory and Nephrology division. The assay took 6.0 minute per sample to complete. This allowed for 5 patient clearance studies per day. Samples were analysed resulted the same day.

Conclusion: A sensitive, precise with a wide dynamic range for LC/MS/MS-based plasma Iohexol assay was developed. There is minimal sample preparation step (dilution and centrifugation) prior to injection. The assay was recently introduced into clinical service and data is being analysed on patients' outcomes and comparison with conventional methods of GFR estimation.

B-105

Moving from Helium to Hydrogen Carrier Gas: The Next Generation of Toxic Alcohols Testing

J. Buse, R. Shyne, Q. Chi, D. Affleck, D. Duce, I. Seiden-Long. *Alberta Public Laboratories, Calgary, AB, Canada*

Background: Rapid identification and quantification of toxic alcohols and glycols provides physicians with information that guides patient management thus reducing patient morbidity and mortality. GC is the most sensitive and specific method available for measuring these analytes. Hydrogen applications have demonstrated the ability to reduce analysis time by at least 25% in comparison to helium. The combination of hydrogen and nitrogen provides an attractive alternative to helium. Both are renewable gasses which can be generated on-site as required rather than purchased in tanks.

Objective: Development of a Gas Chromatography (GC), Flame Ionization Detection (FID), liquid injection method using hydrogen as a carrier gas that combines analysis of alcohols (methanol, ethanol, isopropanol, acetone) and glycols (ethylene and propylene glycol) in a single method.

Methodology: Samples were deproteinized by adding 400 µL of acetonitrile containing internal standards (10 mmol/L N-propyl alcohol for volatile alcohols and 2.5 mmol/L 1,2-butanediol for glycols) to 200 µL of calibrator, QC, or patient specimen, followed by brief vortex mixing and centrifugation at 10,000 ×g for two minutes. GC-FID analysis using hydrogen carrier gas and nitrogen for makeup gas utilized an Agilent 7890 system equipped with Agilent 7683 liquid autosampler. Chromatographic separation was achieved on a 30 m × 530 µm RTX-200 fused silica column (Restek, 15085, Brockville, Canada) with a deactivated fused silica (10 m × 530 µm) guard column (Agilent, 160-2535-10). The method validation included repeatability, recovery, carryover, linearity, lower limit of quantification (LLOQ), accuracy, and selectivity.

Results: The 8.3 minutes required for analysis of a single sample (injection to injection) achieved a 6.7 minute time savings per sample over a previously reported method using Helium carrier gas with no loss in resolution. Within run variability was ≤1.4% and between run variability was ≤6.8% for all analytes. Analyte recovery was determined in triplicate at four concentrations (5, 12.5, 25 and 50 mmol/L) determined to be 100% within a 95% confidence interval. Assessment of carryover was negligible for all but ethylene glycol at 2.1%, which is a 4 fold reduction from the previous Helium method. The LLOQ was 1 mmol/L for all analytes, while the upper range of linearity was 120 mmol/L for methanol, ethanol and isopropanol, 100 mmol/L for acetone and 50 mmol/L for ethylene glycol. All analytes were within +/- 13.8 % of the "All methods mean" for analysis of CAP EQA specimens. Interference analysis demonstrated that toluene co-elutes with ethylene glycol and can lead to a false positive, while benzene, xylene and 1,3 butanediol co-elute with the internal standard 1,2 butanediol and can lead to a reduction in the reported concentration of ethylene glycol.

Conclusions: The GC-FID hydrogen carrier gas method simultaneously quantified methanol, ethanol isopropanol, acetone and ethylene and propylene glycol in a single method achieving a 45% reduction in the time of

analysis. This benefits patient care through a reduction in turnaround time and provides a cost savings to the laboratory.

B-106

Measurement of Monosialogangliosides in GM3 Synthase Deficiency Patient Plasma by a Novel UPLC/MS/MS Assay

R. Wei[1], Q. Huang[1], H. Wang[2], A. Zhou[1]. [1]*Cleveland State University, Cleveland, OH,* [2]*DDS Clinic, Center for Special Needs Children, Cleveland, OH*

Introduction: Gangliosides are a family of glycosphingolipids, abundantly present in the central nervous system of living organisms. Ganglioside GM3 synthase is the key enzyme involved in the initial stages of the biosynthesis of gangliosides. Its deficiency results in a rare metabolic disorder. The clinical manifestations of affected infants include severe irritability, failure to thrive, developmental stagnation, cortical blindness, profound intellectual disability and intractable seizures. Recently we have been conducting a clinical trial to treat this disorder by supplementing extra gangliosides in the formula for the patients. To evaluate the effectiveness of the treatment, the levels of gangliosides in the plasma of the patients were determined by a rapid and sensitive LC-MS/MS method developed in the lab.

Method: Plasma samples from patients with ganglioside GM3 synthase deficiency (GSD) were stored at -20°C before analyses. The prepared plasma samples underwent a protein precipitation procedure by using methanol and chloroform. The analytes and internal standards were then derivatized with 2-(2-Pyridilamino)-ethylamine (PAEA) & 4-(4, 6-Dimethoxy-1, 3, 5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM). The samples were then purified using liquid-liquid partitioning. Samples were analyzed using a Shimadzu Nexera UHPLC system coupled with an AB Sciex Qtrap 5500 mass spectrometer, which was operated in the negative ESI mode. The chromatographic separation was achieved on a Kinetex-C8 UPLC column. A MRM mode was used for quantitation of GM1, GM2, GM3. The LC-MS/MS system was controlled by AB Sciex Analyst® software (version 1.6.3).

Results and Conclusion: GM1 (2D3-GM1), GM2 (2D3-GM1), and GM3 (2D3-GM3) are used as the internal standards. Linear response functions were established in the concentration range of 10.0-2000 ng/ml for GM1 and GM2, and 80.0-1600 ng/ml for GM3, respectively, with correlation coefficients > 0.99.

The method was validated according to the US-FDA guidance for bioanalytical method validation using pooled normal human plasma from Innovative Research, Inc.

It was successfully applied to measure the levels of gangliosides in plasma samples from different sources, including unaffected adults, GSD heterozygous carriers, GSD patients with or without gangliosides fortified formula. Different levels of monosialoganliosides were detected by using the method, indicating oral ganglioside supplement might be a potent treatment for delaying onset and relieving symptoms for GSD patients.

B-107

Ethyl Glucuronide Quantification in Clinical Urine Specimens: Proof of Concept of a Semi-Automated Direct HILIC-HR/MS (Orbitrap) Protocol

H. Guo, E. Wong, C. Stefan. *Centre for Addiction and Mental Health, Toronto, ON, Canada*

Background: Ethyl glucuronide (EtG) is a biomarker of recent alcohol consumption. Its determination in urine specimens is increasingly used in clinical medicine, targeting patients waiting for a liver transplant and/or patients treated in addiction clinics. However, its testing by clinical laboratories is not standardized. Vendor-developed immunoassays can be used for screening, but confirmation requires mass spectrometry-based protocols. Various low resolution GC-MS or LC-MS protocols, with or without solid phase extraction, have been previously published. However, finding suitable chromatographic columns and/or elution conditions has proven challenging due to the highly polar nature of EtG. In addition, the similar fragmentation pattern with other glucuronides when using triple quadrupole mass spectrometry may cause high interferences. Such challenges have prompted us to investigate a HILIC (hydrophilic column)-HR/MS (Orbitrap) protocol for direct determination of EtG in clinical urine specimens

Methods: The Perkin Elmers JANUS automatic liquid handling system was used to prepare the urine specimens: 900 µL Acetonitrile and 50uL EtG-D5 internal standard were added to 50uL urine sample. Chromatography was carried on Agilent's Poroshell 120 HILIC-Z column (2.1 x100 mm, 2.7 µm) using the UHPLC Accela system (ThermoFisher) with a 8-min ACN gradient and 0.5mL/min flow rate. To optimize EtG ionization and/or binding to the column three elution conditions were assessed 10mM Ammonium Bicarbonate, 20mM Ammonium formate and 20mM Ammonium Acetate. To optimize the Q-Exactive (ThermoFisher) high resolution mass spectrometry (HR/MS) acquisition method we investigated Selected Ion Monitoring (SIM) vs. Parallel Reaction Monitoring (PRM) mode. Assay linearity was checked up to 5000 ng/mL; 50 patient specimens and two QC levels at 300 and 600 ng/mL were used for the initial assessment of the proposed conditions.

Results: We found that 10mM Ammonium Bicarbonate mobile phase yielded the highest EtG peak intensity and we used this condition to optimize the HR/MS acquisition method. Based on QC data (triplicates) and the patient specimen data, we estimate that the SIM (high resolution parent ion) acquisition method performed better than the PRM (three high resolution fragment ions) in our HR/MS system, particularly at the lower EtG concentrations (less than 600 ng/mL). The assay was linear up to 5000 ng/mL (8 calibration points, $r^2 = 0.9997$, % difference of each calibration point less than 8.2%).

Conclusion: We investigated a direct EtG quantification protocol in urine specimens. We found that a HILIC-HR/MS (Orbitrap) methodology with SIM acquisition mode warrants consideration for further validation and adoption in a clinical laboratory setting.

B-108

LC-MS/MS Analysis of Endogenous Cannabinoid Plasma Concentrations

M. Hoffman, T. Marcotte, R. Fitzgerald. *UC San Diego, San Diego, CA*

Background: The discovery of cannabinoid (CB) receptors and the growing popularity of cannabis use has sparked interest in understanding the biological function of CB receptors' endogenous ligands, or endocannabinoids (eCBs). The physiological role of two eCBs, anandamide (AEA) and 2-arachidonoyl glycerol (2-AG), has been best described. In addition, THC and CBD have been identified as mimetic phytocannabinoids capable of interacting with CB receptors and, therefore, impact important physiological processes. Widespread recreational and medical use of cannabis and CBD products underscores the importance of understanding how THC and CBD exposure effects the endocannabinoid system. To address this, liquid chromatography - multiple reaction monitoring mass spectrometry (LC-MRM) assays have been developed to measure AEA and 2-AG in patient plasma. **Methods:** Detection of the eCBs was optimized by direct infusion ESI-MS/MS (Waters, TQ-S micro) of pure standard compounds (Cayman). Both compounds performed best in positive ion mode and at least two precursor and product ion pairs (quantifier and qualifier ions) were selected to monitor each compound. Cone voltage and collision energy optimization was performed for ion transitions monitored. Separation of analytes was achieved using reversed-phase liquid chromatography on a Waters Acquity UPLC equipped with a BEH C18 column (2.1 ID x 50 mm, 1.7 um particles) and mobile phases A and B consisting of 5 mM ammonium formate in water with 0.1% formic acid and acetonitrile with 0.1% formic, respectively. A 3.5 min gradient was used with a total run time of 5 min per sample. In addition, the eCB LC-MRM assays are compatible with the previously developed cannabinoid assay panel targeting 10 metabolites and can be combined to quantify all eCBs and cannabinoids in a single analysis. Pre-analytical sample processing was optimized to reduce AEA degradation and isomerization of 2-AG to 1-AG. Heparinized patient plasma was immediately centrifuged following collection and frozen (-20° C) until preparation for LC-MS/MS analysis. Briefly, plasma samples (200 uL) spiked with deuterated internal standards, AEA-d_8 and 2-AG-d_8 (Cayman), and a serine protease inhibitor (PMSF) are prepared using protein precipitation followed by sample clean-up with solid phase extraction. **Results:** Assay linearity was determined for each analyte using an eight point calibration curve. The linear ranges for AEA and 2-AG quantification in plasma are 0.05 to 50 ng/mL and 0.125 to 50 ng/mL, respectively. High and low concentration samples (3.125 ng/mL and 0.3125 ng/mL) were analyzed to assess accuracy and precision. The observable bias for the high and low spiked samples were between -5.6 and 11.7% for AEA and -17 and 15.9% for 2-AG in a 5 day study. In addition, stability in the auto-sampler was assessed at 24, 48, and 96 hours. Results demonstrate that AEA and 2-AG are stable in the LC auto-sampler for up to 24 hours. Sample carryover was evaluated and none observed following injection of the highest calibrator (50 ng/mL). **Conclusion:** These LC-MRM assays measuring AEA and 2-AG will be applied to patient plasma in a clinical trial assessing the therapeutic efficacy and safety of CBD therapy.

B-109

Quantitation of 15 Cannabinoids by LC-MS/MS

P. Nguyen, L. Cao, J. Chung, M. Nguyen, P. Audon, M. Bach, T. Nguyen, A. San Antonio, A. Nguyen, D. Bach. *Bach Diagnostics, Santa Ana, CA*

Background: With Marijuana being legalized in more states recently, there has been a greater need for methods for detecting and quantifying potency of cannabinoids. States have developed guidelines regarding Cannabinoid control and testing. This paper presents a chromatography mass spectrometry method for quantification of Cannabinol, Cannabigerol (CBG), Cannabicyclol (CBL), Cannabidiol, Cannabidivarinic Acid (CBDVA), Cannabichromene (CBC), Cannabidiolic acid (CBDA), Cannabigerolic acid (CBGA), Cannabinolic Acid (CBNA), Cannabichromenic Acid (CBCA), Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV), 11-hydroxy-delta-9-THC, Delta-8-THC, and Delta-9-THC in plant extract. The method is used to identify different cannabinoid concentrations within marijuana products in order to determine accuracy of cannabinoid ingredients within commercial or medical marijuana products.

Methods: Analytical Standards for Cannabinol, Cannabigerol (CBG), Cannabicyclol (CBL), Cannabidiol, Cannabidivarinic Acid (CBDVA), Cannabichromene (CBC), Cannabidiolic acid (CBDA), Cannabigerolic acid (CBGA), Cannabinolic Acid (CBNA), Cannabichromenic Acid (CBCA), Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV), 11-hydroxy-delta-9-THC, Delta-8-THC, and Delta-9-THC were purchased from Cerriliant. Our method uses Omni International Bead Ruptor Elite and 10mg WAX 55-65uM+ 40mg Salt during sample preparation. Cannabinoid separation is performed using Shimadzu 20A Prominence LC. Kinetex Biphenyl 100 Å Column (50mm x 3.0mm, 2.6 um) is used to obtain separation and resolution of the cannabinoids. Mobile Phase A consist of 10mM Ammonium Formate in Water and mobile phase B consist of 0.1% Formic Acid in Methanol. Cannabinoid Samples are analyzed on ABSciex API 4000. Analyst software 1.6.3 is used to generate and quantify results.

Results: The Linear Quantifiable range is 20 ng/mL to 10000 ng/mL with a correlation coefficient greater than 0.99. The precision for all cannabinoids were less than 15% CV. The accuracy for all Cannabinoids were less than 15% CV.

Conclusion: The method was successful in separation and quantification of individual peaks for of Cannabinol, Cannabigerol (CBG), Cannabicyclol (CBL), Cannabidiol, Cannabidivarinic Acid (CBDVA), Cannabichromene (CBC), Cannabidiolic acid (CBDA), Cannabigerolic acid (CBGA), Cannabinolic Acid (CBNA), Cannabichromenic Acid (CBCA), Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV), 11-hydroxy-delta-9-THC, Delta-8-THC, and Delta-9-THC with acceptable resolution, accuracy, precision, and linearity.

B-110

Dried Urine Analysis of Gadolinium, Thallium, and Uranium by ICP-MS with an Emphasis on Inter-Assay Stability of Samples Kept at Room Temperature

T. Zava. *ZRT Laboratory, Beaverton, OR*

Background: Gadolinium, thallium, and uranium are non-essential heavy metals that are normally found at part-per-trillion levels in urine. Exposure can come from natural and anthropogenic sources, including industrial pollution, well water, and soil. Gadolinium based contrast agents (GBCAs) used during MRIs are the primary source of gadolinium exposure. Rare and toxic elemental analysis plays an important role in monitoring the heath of individuals and populations around the world. Inductively coupled plasma mass spectrometry (ICP-MS) allows for the precise quantification of multiple elements simultaneously while maintaining sensitivity and specificity. Dried urine on filter paper is simple to collect, can be shipped without preservatives at room temperature, uses minimal storage space, is adaptable for automated assays, and is therefore ideal for at-home and remote collections and large-scale health surveys. We aimed to validate an assay for, and show the stability of, gadolinium, thallium and uranium from urine collected on filter paper.

Materials and Methods: The dried urine gadolinium, thallium and uranium assay was developed using Whatman 903 filter paper for sample collection and a Perkin Elmer NexION 300D ICP-MS for analysis. Four 6-mm punches of dried urine were extracted in 96-well fritted filter blocks using a 650 µL mixture of dilute nitric acid, triton-X, and indium as an internal standard. Our prior work on essential and toxic elements in dried urine and blood spot made use of the dynamic reaction cell in the NexION 300D, but the sensitivity required for the dried urine gadolinium, thallium, and uranium assay requires analysis in standard mode for low part-per-trillion analysis. Micro-flow pumps along with a low volume nebulizer helped make effective use of the small sample volume.

Results: Accuracy of the method was assessed by testing trace element urine controls from BioRad, ClinChek, and Seronorm for thallium once dried on filter paper. Since external urine controls for gadolinium and uranium are not available, a spiked liquid urine sample was sent to a clinical reference laboratory and simultaneously run as a dried urine sample for comparison. Recovery was demonstrated using urine samples with a known concentration of analyte; acceptable recoveries of 91-117% (mean 105%) were obtained. Linearity was assessed by diluting samples and comparing results to expected concentrations and was excellent across the assay range. Limits of quantification were based on analysis of blank and low-level samples and were in the low to mid part-per-trillion range. Intra-assay precision was based on 20 sample replicates, and the coeffient of variation was <2.2% for all analytes (mean 1.1%). Inter-assay precision was tested during 12 sample runs over 40 days keeping samples at room temperature to replicate conditions of collection and transport in areas without refrigeration. The coeffient of variation for inter-assay precision was <14.6% for all analytes (mean 7.8%).

Conclusions: Dried urine analysis of gadolinium, thallium and uranium using ICP-MS was successfully validated. Demonstrated stability of elements in samples dried on filter paper allows for at-home and remote collections without access to refrigeration, as samples can easily be collected, transported, stored, and analyzed without the use of preservatives for over a month.

Wednesday, August 7, 2019

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

Molecular Pathology

B-111

Elucidating the Relation Between MGMT and ABCG2 Genes Promoter Methylation Status in Advanced Breast Cancer Patients and Response to Cyclophosphamide - Doxorubicin Based Therapeutic Regimen

O. H. Elgaddar, S. A. Aglan, A. Zaki, H. El Sheredy, A. Sobhy. *Medical Research Institute, Alexandria University, Alexandria, Egypt*

Background and Objective: Breast cancer is the leading cause of cancer deaths in developing countries and the second in developed ones. Many factors play important roles in prognosis and clinical management decisions, including: patient's age, tumor size, grade, stage and molecular subtypes. Several guidelines have adopted molecular profiling of breast tumors to aid in staging and selecting patients for chemotherapeutic treatment. Epigenetic modification plays an important role in the pathogenesis of cancer and responsiveness to therapeutic regimens. Selective CpG island methylation is an important epigenetic modification that occurs at genes promoters involved in DNA repair and tumor suppression leading to their silencing and subsequently enhancement of tumor growth. MGMT is a direct DNA repair gene that is ubiquitously expressed. MGMT promoter methylation had been correlated with low expression of its protein and was found to have a predictive value for the response of some tumors to chemotherapeutic regimens.

ABCG2, an ATP binding cassette membrane transporter, is responsible for the export of compounds outside the cell. Its over expression had been linked to chemoresistance as well. Our work aimed at studying the predictive role of MGMT and ABCG2 promoter methylation in the response of breast cancer patients receiving doxorubicin-cyclophosphamide regimens.

Methods: Forty three female patients with advanced breast cancer (stage III and IV) were included in the study assessed for response to chemotherapeutic drugs: AC, FAC and Taxane. They were sub grouped according to response into **responders:** patients who achieved complete or partial response and **non-responders:** patients who had a stable or progressive disease course according to RECIST criteria version. Tumor grading was applied to tumor tissue sample and scored according to Nottingham grading system and staging of patients was done at presentation according to the American Joint Committee on Cancer (AJCC). Molecular subtypes were evaluated using immunohistochemistry assay and grouped into luminal (A and B), triple negative (TN) and HER2 expressing tumor. Methylation analysis of MGMT and ABCG2 genes was performed on formalin fixed paraffin embedded breast cancer tissues, using methyl specific PCR (MSP) technique.

Results: There was no significant association between age and response to any regimen/drug (AC-FAC-Taxane) alone or combined. We examined the predictive effect of grade and molecular subtypes on response to doxorubicin- cyclophosphamide based regimen, but no association awas found between them and the response to different regimens/drugs. Regarding the tumor stage, stage III was statistically associated with response to different regimens/drugs (AC-FAC-Taxane) alone and combined. No association was observed between MGMT and /or ABCG2 methylation and age, molecular subtype, grade and stage. Moreover, no correlation was found between the methylation of both genes, however, a significant association of unmethylation of both genes combined with response to AC followed by Taxane therapy, was detected.

Conclusion: Stage is the most important independent factor for prediction of response to doxorubicin-cyclophosphamide based therapy. Moreover, Methylation event of the promoters of MGMT and ABCG2 combined could be a potential bad prognostic factor for response to AC/Taxane therapy and might be considered as a predictive biomarker prior to administration of that regimen.

B-112

Association Between ApoE4 Genotype and Cognitive Performance in Persons with Subjective Cognitive Complaints

E. Nah[1], S. Cho[2], S. Kim[2], H. Cho[3]. [1]*Department of Laboratory Medicine and Health Promotion Research Institute, Korea Association of Heath Promotion, Seoul, Korea, Republic of,* [2]*Korea Association of Heath Promotion, Seoul, Korea, Republic of,* [3]*MEDIcheck LAB, Korea association of Heath Promotion, Cheongju, Korea, Republic of*

Background: The apolipoprotein E epsilon 4 (ApoE4) allele is the strongest genetic risk factor for Alzheimer's disease and is also of interest in its role as a risk factor for lower cognitive performance in cognitively intact subjects. The aim of this study was to determine the association between the ApoE4 allelic status and the cognitive function of persons with subjective cognitive complaints.

Methods: A retrospective cross-sectional study was conducted with 1,107 examinees with subjective cognitive complaints who underwent both medical and cognitive testing including ApoE genotyping as a part of a health checkup at two health-promotion centers in two Korean cities between January 2016 and December 2017. ApoE was genotyped as recommended in the manufacturer's instructions using the Seeplex ApoE ACE Genotyping (Seegene, Seoul, Korea). Cognitive function was assessed using the Korean version of Mini-Mental State Examination (K-MMSE). All of the subjects were classified according to the ApoE4 status into non-carriers, heterozygotes, and homozygotes. The unadjusted and adjusted odds ratios (ORs) were estimated using logistic regression for assessing the relationship between the ApoE4 genotype and cognitive impairment. ANOVA was used to compare the cognitive domain scores according to the ApoE4 status.

Results: The ApoE4 allele frequency was 10.6%. ApoE4 homozygosity was significantly associated with a lower K-MMSE score (≤23) (OR=4.605, 95% CI=1.301-16.305) compared to ApoE4 non-carriers, and this association remained after adjusting for age, sex, body mass index, blood pressure, blood lipid levels, and fasting blood glucose level. The scores in the temporal orientation and recall domains were significantly lower in ApoE4 heterozygotes than in ApoE4 non-carriers ($P<0.05$).

Conclusion: ApoE4 homozygosity was associated with cognitive impairment in cognitively intact Korean with subjective cognitive complaints.

B-113

Core Body Temperature, and Not Precipitating Activity, are Associated with Measures of Exertional Heat Stroke Severity in a US Military Population; A Retrospective Study

M. Ward[1], M. King[2], L. Leon[2]. [1]*University of Maryland School of Medicine, Baltimore, MD,* [2]*US Army Research Institute of Environmental Medicine, Natick, MA*

Background: Exertional Heat Stroke (EHS) is a serious military and civilian medical problem. The influence of precipitating factors, specifically activities or exercises such as running or load-bearing marches (ruck marches) on EHS patient presentation, remains unclear. Clinical biomarkers of organ injury are currently the best and only measure of injury severity, recovery and physical readiness following EHS.

Purpose: The primary objective of this study addressed whether EHS presentation, [maximal recorded core body temperature (TcMAX)] or activity at the time of collapse (precipitating activity) were associated with subsequent measures of liver and kidney dysfunction, as assessed by serum analytes [creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Cr)].

Methods: This study included a retrospective cohort of EHS patients identified based on ICD-9/10 code diagnosis for Heat Stroke (ICD-9, 992.0; ICD-10, T67.0XXA). Patients were seen at the Womack Army Community Hospital, Fort Bragg, NC from 2010-2015. Electronic health records were obtained from the Armed Forces Health Longitudinal Technology Application (AHLTA), and Composite Health Care System (CHCS). The patient population was parsed into two sets of sub-groups based on (i) precipitating activity at the time of collapse (RUN = running, RUCK = ruck marching) or (ii) TcMAX at the time of presentation, patients in each group presenting with either a lower or higher TcMAX (lower, '<106°F'; or higher, '≥106°F'). Clinical variables are presented as medians for non-parametric data (CK, ALT, AST) and means for parametric data (TcMAX, Cr, BUN).

Results: 177 EHS patient records met the study inclusion criteria. The RUN group presented with a higher TcMAX than the RUCK group (105.9 vs 104.7°F; p=0.0045). However, only select serum enzymes showed significant differences, including ALT and AST (ALT, p<0.0001; AST, p=0.0035). The higher (>=106°F) TcMAX group had

correspondingly higher serum analytes as compared to the lower (<106°F) TcMAX group, for all analytes included in this analysis; CK (676 vs 1086 IU/L, p=0.0252), AST (50 vs 132 IU/L, p<0.0001), ALT (49 vs 129 IU/L, p<0.0001), Cr (1.29 vs 1.633 mg/dL, p<0.0001) and BUN (17.95 vs 20.1 mg/dL, p=0.0032).

Conclusions: These results suggest that higher TcMAX at EHS presentation is associated with EHS severity, as assessed by higher markers of liver and kidney dysfunction. This result challenges current paradigms in sports medicine, that core temperature (TcMAX) is not a relevant indicator of heat injury severity.

Author views not official US Army or DoD policy

B-114

Increasing Reliability of Microbiome Diagnostics of GI Disease by Sample Preservation of Stool and Automated Unbiased Nucleic Acid Extraction

K. Lin, L. Basilio, S. Tang, S. Forman, R. Kemp, X. Jia. *Zymo Research Corp, Irvine, CA*

Background: The gut microbiota has long been associated with GI diseases including, Crohn's disease, ulcerative colitis, and inflammatory bowel disease. Understanding the gut microbiota holds promise for earlier clinical diagnosis of these types of diseases. However, diagnostic success is predicated on accurate detection of microbes in the stool and oftentimes sample degradation can occur due to improper storage which leads to biased results. To combat this, we evaluated a sample collection medium that preserves genetic profiles, inactivates pathogens, and is suitable for direct automated nucleic acid extraction.

Methods: Human stool was stored in preservation medium at ambient temperatures versus unprotected samples and were subjected to repeated freeze thawing at -80°C. After bead-beating homogenization, DNA was extracted using an automated microbiome workflow on a Tecan Fluent. Microbial profiles were analyzed using 16S rRNA gene sequencing on Illumina MiSeq targeting the V3-V4 region. Additionally, a mock microbial standard of various gram positive/negative bacteria and yeast species were used to test the performance of various extraction methods to determine efficiency of lysis.

Results: Microbial composition in preserved stool was unchanged up to 1 month and was unaffected by freeze thaw (up to 10 cycles). Unprotected samples experienced a shift in microbial profiles in as little as one day with unaccounted microbial growth. Overall, there was a complete loss of Bacteroides and significant increase in Actinobacteria in as little as 5 freeze thaw cycles. A majority of extraction methods also revealed a bias toward gram negative species that portrayed a skewed representation of the microbiome.

Conclusion: Microbial profiles remained consistent at ambient temperatures when stool was stored in preservation medium. Furthermore, the preservation medium facilitated nucleic acid extraction and was amenable for automated processing on various instrumentation and chemistries.

B-115

Circulating MiR-373 but not MiR-210 as a New Promising Preoperative Predictor of Response to Superselective Transarterial Chemoembolization in Egyptian Patients with Hepatocellular Carcinoma on top of Hepatitis C Virus Infection

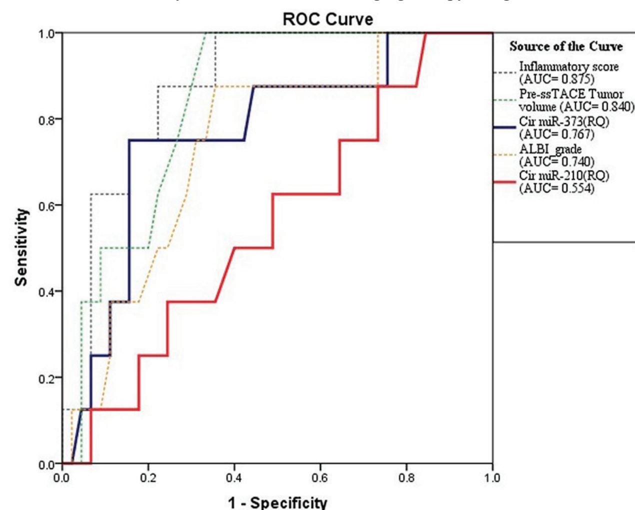
A. Kamel[1], M. Zaki[1], A. Tork[1], R. Abo Elwafa[2], O. Elaassar[2], O. A. Abdelkarem[1]. [1]*Medical Research Institute, Alex, Egypt,* [2]*Faculty of Medicine, Alexandria, Egypt*

Background: Superselective transarterial chemoembolization (ssTACE) has emerged as a bridging therapy for early stages of hepatocellular carcinoma (HCC) awaiting liver transplantation. The study aimed at assessing expression profiles of circulating pre-ssTACE miR-210 and miR-373 as potential predictors of response to (ss)TACE in Egyptian HCC patients on top of chronic hepatitis-C virus (HCV) infection who were candidates for bridging therapy by this treatment modality.

Methods: Fifty-three HCV infected HCC patients awaiting liver transplantation referred for ssTACE and after 3 months follow up period, response was evaluated based on modified response evaluation criteria in solid tumors (mRECIST) which resulted in 45 responders and 8 non-responders . Pre and post-ssTACE tumor volume and viability were assessed using computerized tomography (CT) scan. Circulating pre-ssTACE miR-210 and miR-373 expressions were determined using real time quantitative polymerase chain reaction.

Results: Circulating pre-ssTACE miR-373 but not miR-210, was significantly higher in non-responders compared to responders. Receiver operating characteristics (ROC) curve analysis of pre-ssTACE miR-373, pre-ssTACE tumor volume, inflammatory score and albumin bilirubin grade revealed highest sensitivity (100%) for pre-ssTACE tumor volume (cutoff >11.49 cm^3) and highest specificity for pre-ssTACE miR-373 (84.44%) (cutoff >1.46 fold change) to discriminate responders from non-responders. A combined ROC curve series approach starting with pre-ssTACE tumor volume followed by miR-373, achieved an overall sensitivity of 75% and a specificity of 97.8%. Multivariate logistic regression revealed pre ssTACE miR-373 as a significant independent predictor of response to ssTACE even after adjusting for pre ssTACE tumor volume. The odds of being a non-responder to ssTACE would increase by 1.054 (105.4%) for every unit increase in circulating miR-373 fold change.

Conclusion: Circulating pre-ssTACE miR-373 could assist as a non-invasive tool for better selection of early HCC candidates for bridging therapy using ssTACE.



B-116

Molecular Analysis of Bacteria, Fungi, and Antibiotic Resistance Genes in Diabetic Foot Ulcers

S. M. Jacobs-Helber[1], M. Beckman[1], R. Mullins[2], S. Fagan[2], Z. Hassan[2], B. Homsombath[2], A. Kastello[2]. [1]*RCA Lab Services/GENETWORx, Glen Allen, VA,* [2]*Joseph M Still Research Foundation, Augusta, GA*

Background: GENETWORx was founded in 2011 on the principle that the combination of laboratory testing and pharmacy based therapeutic recommendations provides a powerful aid to clinicians in pursuit of better patient outcomes. Diabetic foot ulcers (DFU) occur as a comorbidity event caused by diabetes and is often caused by a large mass of bacteria and fungi (biofilm). The traditional method for identifying the components of the wound biofilm is microbiological culture. Culture is limited, however, by the inability of anaerobic or fungal microbes to grow in culture. Molecular analysis can specifically detect the presence of pathogens regardless of their ability to be detected in culture. The purpose of this study is to compare the ability of a PCR based molecular diagnostic test to detect common pathogens with traditional culture.

Methods: GENETWORx has developed a high-throughput PCR based panel, Wound-Genie, that provides quantitation of bacterial and fungal pathogens common in chronic wounds and provides therapeutic options to the physician based on the pathogen and antibiotic resistance genes detected. A single-center, prospective study was performed wherein swab specimens were compared using molecular analysis of bacteria and antibiotic resistance to standard wound culture from suspected infection of diabetic foot ulcers. Samples that were positive for bacteria underwent sensitivity testing. Genomic DNA from wound swab specimens were analyzed for the presence of 18 bacteria as well as Candida, Trichophyton, and Aspergillus fungi and 7 classes of antibiotic resistance genes. Clinical decision making in prescribing antibiotic treatment was made on the basis of culture result and the patient's clinical condition and was not within scope of this study.

Results: Observed organisms from aerobic culture ranged from one to four species and were consistent with bacteria commonly isolated from diabetic foot ulcers at JMSRF. There was general concordance with species type grown in culture compared to molecular analysis: All but one of the 8 specimens identified identical organisms in both methods. Further, the Molecular analysis detected the presence of organisms not

detected by culture in 50 % of the specimens. Molecular analysis defined over 90% of all collected genetic material in each of the 8 wounds tested and detected various common resistance genes, not demonstrated in culture specimens. No fungal species were detected via culture, whereas the molecular analysis detected Candida species in 4 of the 8 specimens.

Conclusion: This study supports a growing set of data that suggest that the current practice of selective systemic treatment of microorganisms that preferentially grow in vitro is likely inaccurate and ineffective and may also increase cost and healing time. Molecular analysis such as the Wound-GENIE panel allows the clinician a single, high-yield test that can rapidly reveal the characteristics of wound biomasses that are well-protected from systemic treatments by EPS and resistance genes to allow targeted local therapy based on the presence of the pathogen and therapeutic options.

B-117

Age Associations with Driver Mutations in Lung Cancer

A. Ahuja, M. Shirazi, A. Saqi, H. Fernandes. *Columbia University Medical Center, New York, NY*

Background: Limited studies have investigated the age and gender associations of driver mutations in patients with lung adenocarcinoma (ADAC). Two studies have correlated the presence of *EGFR* and *MET* mutations individually, at the time of diagnosis, with clinical outcomes. However, comparative data on the relationship between *EGFR*, *KRAS*, and *MET* (exon 14 skipping) driver mutations and age of patients at the time of diagnosis of NSCLC, is lacking. The aim of our work is to compare the association of the common driver mutations in patients with lung adenocarcinoma, in a large cohort of patients whose tumor was sequenced at Columbia University Medical Center.

Materials and Methods: Tumor tissue from 950 patients with lung adenocarcinoma, who underwent next generation sequencing (NGS) between 2015 and 2018, were evaluated in this study. Testing was performed using the Truseq Amplicon panel (Illumina). The median age of patients in the three mutation categories was compared with the Kruskal-Wallis test. The presence of mutations stratified by age groups and the gender associations were determined with the chi-square test.

Results: 206 / 950 patients (21.7%) were identified with *EGFR* mutations, 255 (26.3%) with *KRAS* mutations, and 22 (2.3%) with *MET* exon14 skipping. Median (± SD) age for the three categories respectively were 73 (± 9.9), 71 (± 10.2) and 77 (± 11.3), p= 0.0551 respectively. When stratified by 4 age groups, (<60; 61-70; 71-80; >80), *MET* exon 14 skipping was most prevalent in the 71-80 years age group compared to *KRAS* (p=0.001) or *EGFR* (p=0.009). Among the 61-70 age group, a significant difference was observed between the prevalence of variants in *KRAS* (36.5%) and *EGFR* (27.7%) genes (p=0.045). Graphically, it appears that in patients with lung adenocarcinoma, *KRAS* mutations are harbored in younger patients, followed by *EGFR* and then *MET*. All 3 categories of mutations presented with a higher proportion of females with 68% in *EGFR* category, 58.2% in *KRAS* category, and 72.7% in *MET* category (p= 0.061).

Conclusion: As previously established, *KRAS* mutations were most common in our cohort of patients with lung adenocarcinoma, followed by *EGFR* and *MET*. Mutations in *BRAF* were as prevalent as *MET* variants (~2%). Our data potentially suggests that *KRAS* mutations are more prevalent in lung adenocarcinoma patients, diagnosed at an earlier age. *MET* exon 14 skipping (albeit the small N in *MET*), is generally seen in the older age group with lung cancer. The data also shows a tendency for female to male gender predisposition observed in this cohort of patients (p=0.061).

B-118

Validation of Copy Number Variant Detection for a NuclearEncoded Mitochondrial Genes Next-Generation Sequencing Panel

S. E. Roellinger, N. A. Mistry, E. A. Zimmerman Zuckerman, M. A. Dina, G. Sivasankaran, L. M. Peterson, C. Wang, L. Hasadsri. *Mayo Clinic, Rochester, MN*

Background: The majority of mitochondrial proteins (>98%) are encoded by nuclear genes, and mutations in these contribute to multiple mitochondrial disorders. Our current clinical next-generation sequencing (NGS) assay detects single nucleotide and indel variants in 176 clinically relevant, nuclear-encoded mitochondrial genes. Copy number variant (CNV) detection is being validated to enhance this NGS assay. **Methods:** Library preparation and target enrichment were performed via a specific, custom probe mix designed to the genes of interest (Agilent SureSelect^{XT}). Libraries were sequenced on the Illumina HiSeq 2500 using paired end reads. CLC Bio Genomics Server was used for read alignment and variant calling. Copy number variant detection was performed using PatternCNV (Mayo Clinic). To demonstrate accuracy, we assessed twenty-five samples with CNV events previously detected by microarray-based comparative genomic hybridization (aCGH), and ten anonymous samples with presumed normal copy number in the genes of interest. Precision was assessed using four samples run in triplicate both on the same run and across three separate runs. **Results:** Previously reported CNV events were successfully detected in all twenty-five accuracy samples (100% sensitivity). All ten of the anonymous, presumed normal, samples tested had normal copy number in the genes assessed (100% specificity). Results were 100%, 98.99%, 99.94%, and 99.21% concordant among replicates for the four precision samples, respectively (within and across runs). Discordant results were due to false positive calls (resolution is ongoing). Data quality is comparable between all samples types assessed: whole blood, frozen tissue (e.g., muscle biopsy), and fibroblasts. **Conclusions:** Copy number variant detection by NGS is a robust and valuable complement to the currently offered single nucleotide and indel variant detection included in this 176-gene panel. CNV detection using NGS will be beneficial to aid in the diagnosis of mitochondrial disorders involving nuclear-encoded mitochondrial genes without the need for additional testing platforms such as aCGH.

B-119

Case Study from the Correlation of the Infertility Diagnostic Hypothesis and Recurrent Abortion with the Presence of Pericentric Inversion in the Chromossome 9

A. C. M. Romera, M. Conchon, M. P. Migliavacca, J. M. Fenandes. *DASA, Barueri, Brazil*

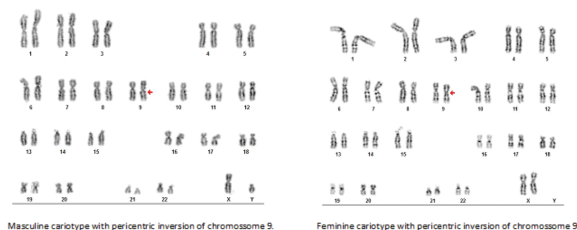
Background: Infertility is caracterized by an incapacity of achieving pregnancy, within a determined period of time or the consecutive flaw of carrying the pregnancy forward. The pericentric inversion of the chromossome 9 is considered to be a common and frequent genetic finding. Its incidence reaches from 1% to 3% of the general population. It's a relative recurrent alteration, being considered a polymorphism without dysmorphic changes, however several studies have shown correlation between infertility or reccurent spontaneous abortion.

Objective: Identify the pericentric inversion of the chromossome 9 in men and women orientated to perform a cariotype for investigation of infertility or recurrent abortion in a clinical brazilian laboratory.

Methods: Evaluate men and women in reproductive age, between 18 and 55 and 18 and 45 years old, respectively, that have undergone cariotype exam in the year 2018 with clinincal diagnosis of infertility or habitual abortion

Results: In 2018 it was researched a total of 4716 cases with pacients in reproductive age, 3546 cases were forwarded to infertility or habitual abortion investigation. Among those cases 60 altered cariotypes were observed (1,69%), subdivided in: infertility cause (1,26%) and habitual abortion (0,43%), presenting the pericentric inversion of chromossome 9.

Conclusion: The altered cariotypes from the presented cases can be recognized as the reason of infertility problems in the population. Although the chromossome 9 inversion is a normal chromossomal abnormality, it is suggestive that such variant should not be ignored. These data can be decisive to determine casual reproductive incapacity and can add important studies of the genetic profile from the brazilian population.



Masculine cariotype with pericentric inversion of chromossome 9. Feminine cariotype with pericentric inversion of chromossome 9.

B-120

Liquid Biopsy for EGFR Mutation Testing in Lung Adenocarcinoma Patients : A 3-Year Experience in a Medical Center of Taiwan

H. Ho, S. Lin, H. Ma, W. Hsu, T. Chou. *Taipei Veterans General Hospital, Taipei, Taiwan*

Background: Lung cancer remains the leading cause of cancer-related mortality worldwide. The development of targeted therapy toward oncogenic driver mutations

has revolutionized the clinical management of non-small cell lung cancer (NSCLC) patients, which account for approximately 80% of all lung cancer cases. Epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) has currently become a standard first-line treatment for advanced NSCLC patients harboring EGFR mutations, especially in East Asia where more than 50% of NSCLC tumors are positive for EGFR mutations. Patients treated with first- and second-generation EGFR-TKIs eventually develop drug resistance, in 50-60% of which emergence of *EGFR* T790M mutation is observed. Osimertinib, the third-generation EGFR-TKI targeting both *EGFR* sensitizing and resistant T790M mutations, is an effective treatment in patients who progress after first-line targeted therapy due to *EGFR* T790M mutation. However, in disease-progressed patients, obtaining tumor tissue for *EGFR* mutation testing through rebiopsy is challenging. Plasma circulating cell-free DNA (cfDNA) is a potential alternative source of tumor DNA. In this study, we report our institutional experience with application of plasma cfDNA for *EGFR* mutation testing in lung adenocarcinoma patients who have received or planned to receive EGFR-TKI treatment.

Methods: From July 2016 to December 2018, one hundred seventy-nine NSCLC patients have performed plasma *EGFR* mutation testing in Taipei Veterans General Hospital, a 3000-bed medical center in Taiwan. The initial *EGFR* mutation status determined from tumor tissue was also reviewed for comparison. *EGFR* mutation analysis in tissue and plasma were conducted using cobas® EGFR Mutation Test v2.

Results: Among 179 patients analyzed, 81.6% (146/179) were positive for *EGFR* mutations in the initial FFPE tumor tissues and after EGFR-TKI treatment, only 49.7% (89/179) had *EGFR* mutations detected in cfDNA, in which 16.8% were L858R mutation, 14% exon 19 deletions, 1.7% G719X, 0.6% exon 20 insertions, 0.6% L861Q combined with G719X, 7.8% exon 19 deletions combined with T790M, and 8.4% L858R combined with T790M. Four of 26 (15.4%) patients negative for *EGFR* mutations in tissue testing were positive in plasma testing. Five of 7 patients (71.4%) failed to detect *EGFR* mutations in FFPE due to poor DNA quality were positive for *EGFR* mutations in plasma testing. These results demonstrated that cfDNA analysis for *EGFR* mutations could be used as an alternative for tissue testing. In 81 cases with *EGFR* mutations detected in both tissue and plasma, there was only a 25.9% upsurge in plasma *EGFR* T790M mutation detection, suggesting that other resistance mechanisms beyond T790M mutation may exist. In addition, we found that cases with *EGFR* mutations determined in tissue testing but disappeared in plasma testing were 50.8% of exon 19 deletions and 36.9% of L858R mutation, implying that the efficacy of EGFR-TKI toward exon 19 deletions is better than that of L858R mutation.

Conclusion: Our experience for application of plasma *EGFR* mutation testing in lung adenocarcinoma patients using cobas® EGFR Mutation Test v2 demonstrates cfDNA analysis could be successfully implemented in clinical settings to rapidly screen and identify resistance mechanisms, allowing early modification of treatment strategies in NSCLC management.

B-121

Evaluation of Minimally-Invasive Detection Assays for Pharmacogenetics Applications

M. A. Pereira[1], J. O. Rodrigues[1], T. M. dos Santos[2], M. S. Gonçalves[2], F. L. O. Marinho[1], D. Zauli[1]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Molecular Genetics Division), Belo Horizonte, Brazil*

Background: The Cytochrome P450 2D6 (*CYP2D6*) gene (MIM #124030) is one of the most polymorphic pharmacogenes with 119 haplotypes reported up to date by the Pharmacogene Variation Consortium (PharmVar) database. Due to this genetic polymorphism, *CYP2D6* exhibit notable inter-individual variability in enzyme activity resulting in a wide variation in drug response that could lead to therapeutic failure. Due to pharmacogenetics importance into clinical practice, we developed an *in-house* test for *CYP2D6* genotyping assignment and phenotype prediction, combining Sanger sequencing and TaqMan Copy Number Assay. Previous results showed that our test was more accurate, sensitive and reliable than the offered by most commercial genotyping kits. **Objective:** Since non-invasive detection assays are preferable, we evaluated the performance of two minimally-invasive sample collection methods - Buccal Swab and Dried Blood Spots (DBS) - as an alternative to blood samples. **Methods:** Genomic DNA was extracted from whole blood, foam tipped swab and DBS samples from 18 individuals. Blood samples were extracted and analyzed twice. Coding sequences and flanking regions of *CYP2D6* gene were amplified and sequenced on ABI 3730 DNA Analyzer. Sequences were analyzed using SeqScape Software v3.0 and compared with the *CYP2D6**1 reference sequence (Accession Number: AY545216.1) to identify polymorphisms. CNV was determined by TaqMan Copy Number Assay (Assay ID: Hs04083572_cn) and RNaseP assay (Assay ID: 4403326) served as the internal control. Relative quantification was performed with CopyCaller® Software v2.1 using the comparative ΔΔCT method. For each individual, the genotype that best represents the set of polymorphisms and CNV data was defined. **Results and Discussion:** DNA yield was different between methodologies and DBS presented the lowest DNA concentration (6.58 ng/µl +- 3.09) and quality/purity ($OD_{A260/A280;}$ 1.55 +- 0.21), followed by foam tipped swab (<20.98 ng/µl +- 7.08; $OD_{A260/A280}$ 1.62 +- 0.11) and blood samples (<85.73 ng/µl +- 25.05; $OD_{A260/A280}$ 1.86 +- 0.06). Some DBS samples presented lower concentration than the recommended (< 5 ng/µl), but this did not interfere with test performance. New minimum DNA concentration validated for CNV assay was 3.5 ng/µl, in agreement with limited sample yield. Sanger sequencing and CNV results were reproducible, presenting 100% of concordance between methods. A high CNV confidence score (> 95%) was observed and most samples presented a very high score (> 99%). **Conclusions:** Both invasive (whole blood) and minimally-invasive (buccal swab and DBS) methods presented similar performance for *CYP2D6* genotype and phenotype prediction and are useful for pharmacogenetics applications, without loss of sensitivity and specificity. This provides an attractive and easy way for patients where whole blood cannot be collected.

B-122

Performance Evaluation of Targeted NGS for *CYP2D6* Genotypnig in Comparison with Sanger Sequencing and MLPA

W. Choi, C. Won, S. Ahn, H. Kim, W. Lee, S. Chun, W. Min. *Asan Medical Center and University of Ulsan College of Medicine, Seoul, Korea, Republic of*

Background: CYP2D6 is an important enzyme involved in the metabolism of about 25% of commonly used drugs. Therefore, accurate analysis of the genotype and phenotype of CYP2D6 can prescribe drugs appropriately for each patient. For this, the guidelines for clinical diagnosis of diagnostic tests are recommending the use of direct sequencing (SS) and multiplex ligation-dependent probe amplification (MLPA). However, testing with next generation sequencing (NGS) can yield massive results faster than SS and MLPA cost-effectively. Therefore, in this study, *CYP2D6* genotype was analyzed by NGS and compared with SS and MLPA results to evaluate NGS performance.

Methods: A total of 1,456 variation sites were screened by NGS for the analysis of *CYP2D6* genotypes by selecting 91 patient samples from hereditary tumor NGS panel. After NGS test, SS and MLPA tests were carried out with residual samples that were frozen and stored. We compared SS and NGS results for 1,456 variation sites and analyzed agreement. The changes of unknown number of copy number variation (CNV) in SS were analyzed by MLPA test results. Then, we compared the differences of the genotype and predicted phenotype of CYP2D6 due to CNV.

Results: All of the 1,456 variation sites were detectable by SS. Compared with NGS results, 1,455 variation sites showed the same result (1,455/1,456), showing different results at one variation site. The MLPA detected unknown alleles (6 cases of *CYP2D6*5* and 5 cases of *CYP2D6*36+*10*) in the SS test and we could observe changes in the *CYP2D6* genotype in 10 samples. However, the predicted phenotype changed from the normal metabolizer to the intermediate metabolizer due to the change of genotype in only 3 samples, and the predicted phenotype did not change in the other 7 samples.

Conclusion: Although the NGS test itself may have several inherent problems, it was able to detect all *CYP2D6* gene variations with high agreement (99.93%) when compared to SS. And we could find 3 cases in which the predicted phenotype was changed due to CNV detected in MLPA test. In the case of tamoxifen, the guidelines published by CPIC suggest it recommended to change the drug dose or replace it with an alternative drug. Even if the predicted phenotype changes, the need to change treatment is scarce because its recommendation level was low. MLPA test could be used to determine the genotype and predicted phenotype of CYP2D6 more accurately. However, there was no difference in the level of the actual clinical application, so the *CYP2D6* genotype could be tested by NGS instead of the SS and MLPA methods.

B-123

Analytical Validation of a Clinical ddPCR Assay for Graft-derived Cell-Free DNA Determination

J. Beck[1], M. Oellerich[2], A. Teubert[3], R. Glaubitz[3], E. Schütz[1]. *[1]Chronix Biomedical, Göttingen, Germany, [2]University Medical Center Göttingen, Göttingen, Germany, [3]amedes Genetics, Hannover, Germany*

Background: Quantification of graft-derived cell-free DNA (GcfDNA) has been widely investigated as new tool to monitor graft integrity. By this time a large body of evidence proves the clinical validity of GcfDNA as biomarker for rejection, whereby the two main testing methods are next-generation sequencing (NGS) or droplet digital

PCR (ddPCR) assays. Before widespread application in clinical practice the analytical performance of the employed methods has to be tested. Here we report the analytical validation of an improved clinical ddPCR method for the quantification of GcfDNA.

Methods: We developed a workflow using 40 preselected SNPs, which are tested in a two-step screening using white-blood cells and plasma from the transplant recipient. The result of the screening are informative assays, of which at least four are used for the quantification of the GcfDNA fraction using ddPCR in all subsequent samples of the same patient. Prior to the quantitative ddPCR the informative assays are preamplified in a multiplex PCR. Analytical performance of the method was assessed in a total of 330 DNA-samples by the following experiments: 1) all 40 assays were run in triplicate on DNA-mixtures with the respective minor allele frequency of 2%; 2) the limits of blank (LoB) were assessed using four different samples each with 10 replicates and evaluated for false-positive allele counts; 3) the limit of detection (LoD) was calculated as LoB+1.645*STDEV of a 0.2% DNA mixture measured in twenty repetitions; 4) lower limit of quantification (LLoQ) was assessed by measuring five different DNA-mixtures (0.1-0.3% MAF) in five repetitions on four different days and three mixtures (0.4-10% MAF) in ten repetitions on the same day; 5) minimum and maximum input amounts of total cfDNA were assessed.

Results: The LoB was 0.1% and the assay reliably quantifies GcfDNA fractions from 0.15% (interassay CV <20%=LoD) to 99%, with an interassay CV of 12% at 0.23% GcfDNA and 10% at 0.32% GcfDNA. At 9.4% GcfDNA the CV was 3%. Importantly, the sensitivity and accuracy of liquid biopsy assays strongly relies on the amount of total cfDNA used as input. Therefore, our method includes a ddPCR that precisely quantifies the amount of extracted total cfDNA and allows tight control of the input amounts in the diagnostic ddPCR that determines the GcfDNA fraction. The minimum amount of total amplifyable cfDNA is set at 4545 haploid genomic copies. Furthermore, the upfront quantification of the total cfDNA per mL plasma can be used to calculate the absolute GcfDNA levels in cp/mL plasma from the fractional abundance. This combined measurement also allows the compensation of fluctuations in the recipient cfDNA levels which would otherwise falsify the fractional GcfDNA values.

Conclusion: Clinical diagnostic assays employing cell-free DNA must be strictly controlled and must be able to reliably quantify even low fractions of the analyte in low total input quantities. The improved ddPCR meets all criteria for a clinical assay for monitoring graft integrity in solid organ transplantation.

B-124

Chromosomal Microarray Analyses as an Initial Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies in a Large Private Laboratory in Brazil

L. T. Galindo[1], A. D. Hristov[1], A. Krepischi[2], S. Costa[2], L. C. Scarpelli[1], F. Milanezi[1], M. P. Migliavacca[1], J. E. Levi[1], G. Campana[1], C. Rosenberg[1]. *[1]DASA- DIAGNOSTICOS DA AMERICA, BARUERI, Brazil, [2]University of São Paulo, São Paulo, Brazil*

Background: In 2010 the International Standard Cytogenomic Array Consortium (ISCA) issued a consensus statement that chromosomal microarray analysis should be considered as the first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. This recommendation was based on the much higher diagnostic yield of chromosomal microarrays compared to classical cytogenetics and since then has been used in the US and most European countries. However, nowadays in Brazil, karyotyping is still the most frequently requested analysis as the initial clinical diagnostic test for these individuals, and health insurance companies do not cover array costs before karyotyping is performed. Here, we report the results of chromosomal microarray analyses of 657 patients with developmental disabilities or congenital abnormalities. To our knowledge, this study albeit modest, is the largest consecutive cohort of Brazilian patients reported so far. We hope this contribution will help implementation of chromosomal microarray analyses as a routine diagnostic test in Brazil. Because the platform used (SNP array) also allows identification of homozygous regions, these results will contribute also to evaluation of consanguinity in developmental disabilities or congenital anomalies in Brazil.

Methods: The patients were referred for chromosomal microarray analyses due to unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies. Chromosome microarray analysis was performed using the Affymetrix 750K SNP platform, according to the manufacture specifications.

Results: from the chromosomal microarray analyses of 657 patients, 121 showed alterations considered pathogenic or likely pathogenic (18.4%). Another 121 patients showed variants of unknown significance (16.7%). The use of SNP arrays allows the identification of uniparental disomy (UPD), but a single case of UPD (from chromosome 15) was detected. On the other hand, 29 patients (4.4%) showed large blocks of homozygosity (> 10 MB), indicating consanguinity among their parents.

Conclusion: This series of chromosomal microarray analyses reported from Brazilian patients shows a diagnostic yield and spectrum of genetic causation compatible with literature reports emanating from the USA and Europe, confirming the superiority of this approach compared to G-banded karyotyping for investigating patients with unexplained developmental delay/intellectual disability, autism spectrum disorders, or multiple congenital anomalies. The use of a SNP array platform uncovers UPD cases, which are not detectable by array-CGH platforms. Additionally, the fact that 4.4% of the patients have consanguineous parents shows that, although consanguineous marriages are not a cultural practice in Brazil, it likely contributes to developmental disabilities and congenital anomalies in the country.

B-125

Retrospective Analysis of Cytogenetic Findings Involving Chromosome 18 and Its Abnormalities on Database of a Cytogenetic Laboratory from DASA, Brazil

A. M. Silva, M. P. Migliavacca, M. Conchon, J. M. Fenandes. *DASA, Barueri, Brazil*

Background: Unbalanced translocation are the exchange of chromosome parts that can result in derivative chromosomes, lost or addition of genes and are the most common cause of miscarriages or children with some deficiency, physical or mental. The deletion of part or the entire short arm of chromosome 18 is a rare chromosomal aberration, with a frequency of 1 to 50.000 live births, and female to male ratio of 3:2. Was first described by Jean de Grouchy in 1963 and around 300 cases have been reported worldwide. Clinical features of this syndrome vary from patient to other and chromosome breakpoints, but most cases present moderate mental retardation, growth deficiency, round face with deformities, along with neurological and dental deformities, being mistaken with Down or Turner´s Syndrome.

Objective: To identify cytogenetic findings involving chromosome 18.

Method: Retrospective analysis of peripheral blood karyotype database of the Cytogenetic laboratory from DASA, between the years of 2017 and 2018.

Results: From 16.811 cases, only 9 cases (0.05%) of children under 15 years old were diagnostic with some abnormality on chromosome 18. The diagnostic hypothesis was Syndrome (Down, Turner´s or Edward´s), dysmorphism, cardiopathy and intellectual deficit. The results (presented on figure 1) were: 1 case of addition (?add(18)(p11.3) - 0.005%); 2 cases of deletion of long arm (?del(18)(q21.3) and del(18)(q?22) - 0,011%); 4 cases of deletion of short arm (del(18)(p11.2) and del(18)(p11.2) - 0,023%) and 2 cases of derivative chromosome (der(18)t(18;18)(p11.2;q?22) and der(15;18)(p10;q10) - 0,011%).

Conclusion: The results demonstrate the small incidence of abnormalities on chromosome 18 findings in karyotype. Most cases reported are due to *de novo* translocations, not inherited, however, parents must be investigated as well in order to find balanced translocations and orientated to genetics counseling. Once the phenotype varies, a multidisciplinary team should be approached to provide a better life quality to the patient and the family.

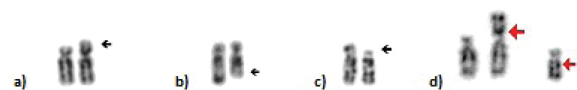


Fig1. a) Addition on short arm, **b)** Deletion of long arm, **c)** Deletion of short arm, **d)** Derivative chromosome due to translocation (15;18) and monosomy of 18p.

B-126

Investigation of Copy Number Variation (CNV) Alterations in Patients with Autism Spectrum Disorder (ASD) in a Brazilian Cohort

M. S. Gonçalves[1], F. K. Marques[2], J. O. Rodrigues[2], A. A. G. Cruz[3], N. D. Poppi[3], A. M. Chami[3], F. G. C. Amorim[3], M. S. Carvalho[3], J. M. Moreira[3], D. Zauli[2]. *[1]Hermes Pardini Institute (Genetics Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [3]Federal University of Minas Gerais, Belo Horizonte, Brazil*

Background: Autism spectrum disorder (ASD) is a complex neurobiological disorder characterized by neuropsychological and behavioral deficit of varying severity. This disorder can exist in isolation or as part of a syndrome in association with additional

features such as developmental delay and other comorbidities. The disease is seen four times more frequently in males than females. ASD is heritable with complex inheritance and genetic heterogeneity, and frequently coexists with other diseases such as intellectual disability, seizure disorders, and fragile-X. Submicroscopic copy number variants (CNVs) may have a causal or susceptibility related role in the heritability of ASD. **Objective**: To investigate the contribution of the CNVs in autism spectrum disorder. **Methods:** The study involved patients from the child psychiatry service under regular follow-up at a hospital in Minas Gerais, Brazil. A child psychiatrist based on clinical criteria of DSM IV/V and ICD-10 performed the diagnosis of ASD. Clinical and demographic data were obtained through medical records and interviews with parents and relatives. Systematic screening for pathogenic mutations was performed by karyotyping, fragile X syndrome testing and targeted MLPA testing. Cytogenetic analysis was performed using conventional G-banding (400-450 band level) from peripheral blood cultures. DNA samples were tested to determine the number of CGG repeats in the FMR1 gene by PCR for investigation of Fragile X syndrome. We evaluated a targeted approach based on multiplex ligation-dependent probe amplification (MLPA) to detect clinically relevant variants for diagnostic testing of ASD patients. MLPA tests were performed with SALSA® MLPA® P245-B1 (ASD related to genetic syndromes) and SALSA® MLPA® P343-C2 (chromosomal regions related to ASD) kits. **Results and discussion:** We analyzed 65 samples of children and adolescents aged 2 to 17 years (mean age 10 ± 3.21 years), being 80% male. The mean gestational age was 29± 6,82 weeks, with 13.8% being premature. History of neonatal jaundice (3,1%) and epilepsy (2.48%) were the most frequent clinical data. The mean maternal age at birth was 29 ± 8.62 years. Pre-eclampsia was reported in 5.58% and gestational diabetes in 1.86%, all in term pregnancies. All patients were evaluated for the presence of dysmorphisms, present in 40% of them. The overall detection rate with the proposed genetic tests was 6.1%. All patients presented normal karyotype. In the study of expansive mutations in FMR1 one male patient presented an allele with 92 repetitions of the CGG triplet (pre-mutation). MLPA P245 kit detected deletion of the RABL2B and SHANK3 genes on 22q13.33 in a female patient, overlapping with a SKANK3 deletion detected by P343-C2 kit. The MLPA P245 kit also detected a deletion on 15q24 in a male patient. **Conclusions:** The identification of genetic abnormalities may contribute to improved diagnosis and potentially influence therapeutic approaches in ASD. Our results suggest that it may be possible to use the screening for Fragile X Syndrome and the MLPA Kits P245-B1 and P343-C1 for a more efficient screening during ASD diagnosed by conventional chromosomal analysis, supporting the knowledge of the literature.

B-127

Tuberous Sclerosis Complex (TSC) Screening using NGS and MLPA in Brazilian Patients

J. O. Rodrigues[1], M. A. Pereira[1], J. P. Silva[1], F. L. O. Marinho[1], R. L. M. Guedes[1], G. R. Franco[2], D. Zauli[1]. [1]*Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil,* [2]*Biochemistry and Immunology Department, UFMG, Belo Horizonte, Brazil*

Background: Tuberous Sclerosis complex (TSC) is a rare, autosomal-dominant disorder caused by genetic variation in at least one of two tumor suppressor genes: *TSC1* (9q34) or *TSC2* (16p13.3). TSC causes hamartomas (non-malignant tumors) growth in multiple organs and presents a broad range of symptoms. Current diagnostic criteria for TSC is based mainly on clinical features, however the identification of a pathogenic variant in TSC genes is sufficient for TSC diagnosis. Next Generation Sequencing (NGS) and Multiplex Ligation-Dependent Probe Amplification (MLPA) have been introduced into DNA diagnostic laboratories for the detection of molecular genetic alterations that are of diagnostic and prognostic significance, such as SNPs and large gene duplications and deletions. **Objective:** Investigate the presence of gene mutations in 28 Brazilian patients with definitive or possible clinical diagnostic of TSC using a target custom sequencing panel and MLPA. **Methodology:** Genomic DNA was extracted from 28 patients from the Brazilian Association of Tuberous Sclerosis (ABET). A custom AmpliSeq™ panel was designed to target coding DNA sequences (*TSC1* exons 3 to 23; *TSC2* exons 2 to 42) and at least 10 bp of flanking introns. Bioinformatics analyses were performed on Torrent Suite™ 5.6 Server and Ion Reporter™ 5.6 software. Variants pathogenicity were assessed according to the ACMG/AMP recommendations. Sanger sequencing was performed to confirm variants classified as pathogenic, likely pathogenic and variant of unknown significance (VUS). Genomic rearrangements (deletions/duplications) were assessed by commercial MLPA kits P124-C2 TSC1 and P046-C1 TSC2 covering all exons from both genes, according to manufacturer's instructions (MRC-Holland). P046-C1 contains an additional probe for *PKD1* gene, adjacent to *TSC*2. **Results and Discussion:** Twenty-four patients (85.7%) were found to have disease-causing mutations, in accordance with previous findings for Brazilian and other populations. Exonic and 10 bp exon/intron boundaries variants were evaluated, representing a total of 54 distinct variants: 9 (16.67%) in *TSC1*, 44 (81.48%) in *TSC2* and one (1.85%) in *PKD1*. Among them, 12 (22.22%) variants were novel and have not been previously reported in databases. Variants were classified using ACMG-AMP criteria representing 11 pathogenic, 10 likely pathogenic and one VUS. Two (7.14%) patients carrying only VUS or (likely) benign variants were considered as no mutations identified. *De novo* mutation analysis was only performed for three families and for other two families only one parent was evaluated, representing three sporadic (one *TSC1* and two *TSC2*) and two familial (one *TSC1* and one *TSC2*) cases. MLPA analysis identified two likely pathogenic heterozygous deletions in *TSC*2, one false-positive (FP) *TSC2* deletion and one VUS heterozygous deletion in *PKD*1 gene. FP result was due to the presence of a frameshift mutation in probe ligation site preventing probes ligation, amplification and detection. Genomic rearrangements were not detected for *TSC1*. Sanger sequencing also detected two NGS FP results in *TSC2* due to strand bias sequencing. **Conclusions:** Our study reinforced the importance of genetic testing in TSC patients and their family members. Combining next-generation sequencing and copy number variation analysis is a comprehensive and useful approach for mutation screening, increasing molecular diagnosis.

B-128

Evaluation of Droplet Digital PCR and PCR/Capillary Electrophoresis Assays for Carrier Screening and Diagnosis of Spinal Muscular Atrophy

M. R. Al-Turkmani, E. G. Hughes, C. E. Griffin, A. C. Mackay, G. J. Tsongalis, J. A. Lefferts. *Dartmouth-Hitchcock Medical Center and Geisel School of Medicine at Dartmouth, Lebanon, NH*

Background: Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by degeneration of alpha motor neurons, leading to muscular weakness and atrophy. SMA is usually caused by a homozygous deletion of exon 7 of the *SMN1* gene. In SMA patients, disease severity correlates with the copy number of a nearby *SMN1* paralog, *SMN2*. SMA carrier screening has recently been recommended to all women who are considering pregnancy or currently pregnant, leading to a significant increase in SMA carrier screening volumes in the clinical laboratory. The objective of this study was to compare two different assays for *SMN1* copy number determination: a droplet digital polymerase chain reaction (ddPCR) assay (Bio-Rad Laboratories, CA) and the Amplidex PCR/capillary electrophoresis (PCR/CE) assay (Asuragen, TX). **Methods:** DNA was extracted from whole blood samples obtained from 69 women subjected to SMA carrier screening and one confirmed SMA patient. Additionally, DNA extracts from the fibroblasts of two SMA carriers and five SMA patients were obtained from the Coriell Institute (NJ) and included in the study. A DNA input of 40 ng was used for both ddPCR and PCR/CE assays. The ddPCR protocol uses primers and florescent probes targeting exon 7 of the *SMN1* and *SMN2* genes as well as a reference gene (*RPP30*). The PCR/CE assay determines *SMN1* copy number by taking the ratio of the *SMN1* amplicon CE peak area relative to that of a co-amplified endogenous control gene. Results were compared to those obtained by multiplex ligation-dependent probe amplification (MLPA) performed by an academic reference laboratory with copy number determination of *SMN1* and *SMN2*. Inter-assay and intra-assay precision was determined using patient samples that had 0, 1, 2 and 3 copies of *SMN1*. **Results:** Among the 69 SMA carrier screening samples, 8 samples had one copy of *SMN1* (SMA carrier status), 48 had two copies, 10 had three copies, and 3 had four copies of *SMN1* as determined by the MLPA method. *SMN1* copy number results for both ddPCR and PCR/CE were in full agreement with MLPA results or expected results for samples extracted from whole blood or fibroblasts. *SMN2* copy number results by MLPA were available for 64 samples and they were 98% concordant (63/64) between the ddPCR and MLPA assays. The one discordant sample had three copies of *SMN2* by ddPCR versus four copies by MLPA. Since that subject had two copies of *SMN1*, this difference in *SMN2* copy number had no clinical significance. Results by ddPCR and PCR/CE were reproducible across multiple runs. **Conclusion:** The evaluated ddPCR and PCR/CE assays for *SMN1* determination showed acceptable accuracy and precision. Their simple and comparable workflow make them both appropriate for SMA testing in the clinical laboratory. Additional *SMN2* copy number determination by the ddPCR assay allows evaluation of disease severity in SMA patients.

B-129

Peripheral Biomarkers as Predictors of Cognitive Decline in an Elderly Population from Brazil

F. L. O. Marinho[1], L. S. Vasconcellos[2], H. C. Guimarães[3], M. T. Barbosa[3], A. L. Teixeira[3], D. Zauli[4], P. Caramelli[3], K. B. Gomes[5]. *[1]Hermes Pardini Institute (Research & Development Division) and Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [2]Departamento de Propedêutica Complementar da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [3]Departamento de Clínica Médica da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [4]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [5]Departamento de Análises Clínicas e Toxicológicas da Universidade Federal de Minas Gerais, Belo Horizonte, Brazil*

Background: Neurodegenerative diseases are related to cognitive, behavioral and motor dysfunction, as well as progressive incapacity. Currently, the diagnosis of neurodegenerative diseases is based on clinical aspect, mainly the relative impact of impairments on daily activities. **Objective:** The aim of this study was to evaluate biochemical markers and their association with cognitive decline in an elderly population aged over 75 years from Caeté/MG (Brazil), included in the Pietà Study - an epidemiological research about the healthy brain aging. **Methods:** A total of 123 voluntaries aged over 75 years underwent a comprehensive medical evaluation, including a brief cognitive screening battery, which was normalized into Z[[Unsupported Character - Codename]]scores, and allowed the calculation of a Global Cognitive Score (GCS). The participants provided blood samples at baseline for the following analyses: hemoglobin, leukocyte, platelets, glucose, triglycerides, total cholesterol, HDL-cholesterol, VLDL-cholesterol, LDL-cholesterol, thyroid-stimulating hormone (TSH), gamma glutamyl transpeptidase (GGT), creatinine, B12 vitamin, cortisol and apolipoprotein E genotyping. In the following year, the subjects were classified as cognitive decliners or not according to their worsening performance in GCS. Data were evaluated using SPSS statistical software v.19. The significance level was p value < 0.05. **Results:** The population comprised 60.2% of women and 39.8% of men. About the cognitive impairment, 54.5% showed decline after one year, while 45.5% presented improvement or maintained the same cognitive capacity. Only triglycerides levels was different between the groups: median = 128 (interquartile range = 79) mg/dL for the group that declined, and 105 (63) mg/dL for non-decliners (p=0.007). **Conclusions:** The results suggest that higher triglycerides levels are associated with cognitive decline in patients aged 75 years or older, and that dyslipidemia could be associated to pathophysiology of dementia processes.

B-130

Evaluation of a Cartridge-Based System for Rapid Detection of *BRAF* and *NRAS* Mutations in Melanoma

M. R. Al-Turkmani, A. C. Mackay, D. C. Green, K. N. Godwin, R. E. LeBlanc, G. J. Tsongalis. *Dartmouth-Hitchcock Medical Center and Geisel School of Medicine at Dartmouth, Lebanon, NH*

Background: Melanoma is the fifth and sixth most common cancer in men and women, respectively and it is responsible for the majority of skin cancer-related deaths. Treatment of melanoma has been revolutionized with the development of effective molecular and immune targeted therapies. Approximately half of unresectable or metastatic melanomas harbor a mutation in *BRAF*, with V600E being the most common. *NRAS* mutations are found in 15 - 20% of melanomas and are typically associated with aggressive disease and poorer outcomes. The objective of this study was to evaluate targeted mutation testing of *BRAF* and *NRAS* in melanoma using the Idylla™ system, which is a fully integrated, cartridge-based system that provides automated sample processing (deparaffinization, tissue digestion and DNA extraction) and real-time PCR-based mutation detection with all reagents included in a single-use cartridge. **Methods**: Twenty-two archived formalin-fixed paraffin-embedded (FFPE) melanoma tissue specimens were tested on the Idylla™ system (Biocartis, Belgium) using the NRAS-BRAF cartridges (Research Use Only). Among these samples, 8 had a mutation in *BRAF,* 6 had a mutation in *NRAS,* and 8 had no mutations in *BRAF* or *NRAS* as determined by previous next-generation sequencing (NGS) testing using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific). For testing, tumor tissue was enriched by macrodissection after the tumor area and neoplastic cellularity were determined by a board certified dermatopathologist to ensure specimens met assay requirements (≥ 10% neoplastic cellularity and 50-60 mm^2 tissue area). Limit of detection was evaluated by testing commercial standards derived from human cell lines that harbor *BRAF* or *NRAS* mutations at 5% variant allele fraction (VAF). Precision was determined by testing 3 patient samples in triplicate. Analytical specificity (interference study) was evaluated by testing samples with high melanin content and samples with mutations in *NRAS* and *BRAF* other than those detectable by the NRAS-BRAF cartridge. **Results:** The Idylla™ system successfully detected mutations in *BRAF* (V600E, V600K, V600R) and *NRAS* (G12C, G12V, G13R, Q61K, Q61R) and results were in full agreement with those obtained previously by NGS. No mutations were detected in the wild-type samples. Results were reproducible and the 5% VAF limit of detection was verified. No interference by melanin or other *NRAS/BRAF* mutations was detected. The system produced results quickly with a turnaround time of approximately 2 hours. **Conclusion:** The Idylla™ system offers rapid and accurate testing of clinically actionable *NRAS* and *BRAF* mutations in melanoma directly from FFPE tissue sections. Its simplicity and ease of use compared to prevailing molecular techniques make it ideal for small centers that lack highly trained staff and infrastructure. It can also complement NGS at larger diagnostic centers by providing rapid turnaround times that benefit time-sensitive decisions in melanoma.

B-131

Investigate microRNA-122 to Identify Liver Injury in Patients with Rhabdomyolysis

Y. Zhang, K. Lynch, A. Wu. *San Francisco General Hospital, San Francisco, CA*

Background: Drug-induced liver injury (DILI) after pharmaceutical treatment or use of recreational drugs is a leading cause of morbidity and mortality. It is important to detect and monitor DILI in a timely and accurate manner. Aminotransferases have been validated as the gold standard biomarkers of liver injury. However, due to their expression in other tissues than liver, the elevation of ALT/AST could also be observed in other clinical conditions, such as rhabdomyolysis. Therefore, developing alternative biomarkers for DILI is meaningful in clinical practice, especially for drug-abuse patients who have high risk to develop rhabdomyolysis and/or DILI. MicroRNA-122 (miR-122) is dominantly (~70% of total miRNA content) and specifically expressed in liver, making it an ideal candidate as a DILI biomarker. We investigated the diagnostic value of miR-122 for DILI in patients with rhabdomyolysis.

Subject Recruitment: Rhabdomyolysis patients with creatine kinase (CK) > 5 x upper limitation of normal (ULN), including 15 males aging 21-73 and 2 females aging 59-65, were recruited at San Francisco General Hospital. 9 out of 17 patients were positive with drug abuse, 1 took atorvastatin, and the other 7 had muscle injury caused by crush/fractures, surgery, seizures, or excessive exertion *etc.* Serum samples (55 in total) were collected at different time points for each patient. Meanwhile, serum samples from 19 healthy subjects and 6 patients with confirmed liver injury were included as reference samples and positive controls respectively and tested at the same time.

Methods: RNA was purified with Trizol LS from 350 μl serum and followed by reverse transcription, and cel-miR-39 (5 fmol, 5 μl) was sparked into each serum sample as the internal control right before RNA purification. The miR-122 level was estimated with quantitative real-time PCR (Taqman MicroRNA assay, miR-122-5p) and calculated relatively to cel-miR-39.

Results: In the rhabdomyolysis patient samples, we observed the remarkable increase in ALT/AST, which was closely correlated with CK. However, miR-122 level in most samples (49 out of 55) were within the reference range, and only 6 out of 55 serum samples from 3 out of 17 patients had miR-122 level (Arbitrary Unit, A.U.) higher than ULN (2.85, calculated as Mean + 2 x SD of reference samples). In serum samples with confirmed liver injury, miR-122 got increased significantly (p<0.01), and the serum level was at least 5 times higher than ULN and could reach 1000 A.U.

Conclusion: MiR-122 in serum does not appear to be interfered with the present of muscle injury. In cases with liver injury, miR-122 could monitor liver injury with high sensitivity comparable to ALT/AST. Moreover, miR-122 could be more specific for liver injury compared to ALT/AST, which is especially meaningful for cases with rhabdomyolysis. We are currently collecting more clinical evidences for further validation.

B-132

Comparison and Validation of Three Bioinformatics Pipelines and QC Programs for Targeted Next Generation Sequencing Panels

N. Yu, J. Lee, Y. Kwon, M. Kong. *Seegene Medical Foundation, Seoul, Korea, Republic of*

Background: Analytical validation of next-generation sequencing (NGS) bioinformatics (BI) pipelines are an essential part for accuracy and consistency in clinical laboratory NGS practice. It is recommended that clinical laboratory offering NGS testing should perform their own validation of BI pipeline for various types of vari-

ants. Here, we evaluated three BI pipeline (one customized and two commercials) to confirm its analytical performance and validated it using reference material and patients' samples. In addition, quality control (QC) indicators were evaluated and compared using associated programs.

Methods: NGS-based targeted sequencing was performed on the reference DNA with 23 pathogenic variants in GM24385 background and samples of patients with 22 variants using hereditary cancer panel (60 genes for germline variants). The reference DNA with 21 pathogenic variants and samples of patients with 24 variants were used for NGS sequencing for validation of BI pipeline in hematologic malignancy gene panels (50 genes for somatic variants). All NGS tests were performed by Illumina Miseq Dx platform. To call variants of the sequenced data, Dxseq (commercial cloud-based analysis system, Dxome, Korea), customized BI pipeline (based GATK 4.0 version), and CLC Genomics Workbench software (CLC Bio, Qiagen, USA) were used together. Some variants were removed or identified by inspecting the NGS data using Integrative Genomics Viewer (IGV). The QC metrics were compared through 2 commercial analysis pipeline and MultiQC sofrware (Science for Life Lab).

Results: As a result, 43 among 44 pathogenic variants composed of various types were found using three pipelines. Interestingly, the one pathogenic variants in which the Alu Y sequence of 343bp size is inserted did not be called by all three pipelines. In this case, we identified it using IGV to find the split reads which were mapped into the other region from break point on reference genome. Therefore, using our integrated analysis method with BI pipelines and IGV can be used to detect numerous types of variants accurately and reliably. In addition, the three QC programs enabled us to select and compare QC metrics that enabled us to effectively evaluate the outcomes.

Conclusion: Acceptable performance in precision, LoD and accuracy was shown by three BI pipeline, in both germline and somatic panels. We confirmed that customized BI pipeline showed a good analytical performance in detecting somatic variations with more than 5% variant allele frequencies in samples of myeloid neoplasm. Other BI tools, Dxseq cloud server system and CLC Genomics Workbench also could be use as alternative tool for analysis of panels in various germline and somatic variants.

B-133

Performance Characteristics of a Workflow for Massive Parallel Sequencing of a Comprehensive Panel of Genes Associated with Clinical Phenotypes in Order to Implement Several Disease-Focused Sub-Panels in a Clinical Laboratory using One Physical Assay

G. Barra, P. Mesquita, T. Santa Rita, R. Jácomo, L. Nery. *Sabin Medicina Diagnóstica, Brasília, Brazil*

Backgound:

Sequencing a comprehensive panel of genes associated with different phenotypes and then bioinformatically analyze disease-focused sub-panels could minimize validation efforts and combine samples with different disease in one assay. Here we use a method-based approach to validate a NGS workflow for 3921 genes from which 228 disease-focused sub-panels could be derived using one physical assay.

Methods:

Whole blood from 16 volunteers was used. DNA was extracted using MagNA Pure 24 (Roche). DNA and sequencing libraries were qualified/quantified using TapeStation4200 (Agilent). Test method included coding regions ±10bp flanking intronic sequences of 3921 genes enriched using Kappa HyperPlus Library Preparation Kit (Roche) and SeqCap EZ inherited disease panel (Roche) and sequenced (2x75-bp Mid Output V2 Reagent) using NextSeq-500 (Illumina) (estimated mean coverage-100X). Read alignment and variant calling were performed with BWA enrichment (BaseSpace Sequence Hub - Illumina). Variant annotation and filtration were performed with Variant Interpreter (Illumina). SNVs and small indels (<5bp) with total-read-depth >20X, variant-allele-depth >8X and variant-read-frequency >20% were analyzed. The test method was performed twice to evaluate the assay inter-run precision (reproducibility) for heterozygous/homozygous variant calls. The reproducibility was number of variants with same results divided by number of unique variants in both runs. All target regions were considered (3921 genes, 54774 genomic regions and 10042113 nucleotides). Reference method was exome sequencing performed using SureSelect Human All-Exon-V7 Exome (Agilent), SureSelectXT Target Enrichment System (Agilent) and sequenced (2x150-bp High Output V2 Reagent) using NextSeq-500 (estimated mean coverage-300X). The above-described bioinformatics pipeline was applied. Only targeted regions shared by both enrichment systems were included (3911 genes, 53980 genomic regions and 9957670 nucleotides). Positive percent agreement (true-positives/true-positives+false-negatives) and analytical positive predictive value (true-positives/true-positives+false-positives) for heterozygous/homozygous variant calls were calculated considering reference method results as true.

Results:

The target mean coverage was 96.8X (85%>50X) and 101X (88%>50X) for the test method first and second run, respectively, and 299.2X (96.6%>50X) for the reference method run. The test method called 7032 SNVs and 181 indels in the first run and 7046 SNVs and 178 indels in the second run. Both runs agreed in 6916 SNVs and 156 indels (116 SNVs and 24 indels and 129 SNVs and 21 indels were called in one run but not in the other) resulting in a reproducibility of 96.56% for SNVs and 77.54% for indels. The test method called 6970 SNVs and 173 indels while the reference method called 7017 SNVs and 211 indels. Both methods agreed in 6722 SNVs and 150 indels positions indicating a positive concordance of 95.8% for SNVs and 70.95% for indels, 248 SNVs and 23 indels were called only by the test method and 295 SNVs and 61 indels were called only the reference method resulting in a technical positive predictive values for the test method of 96.4% for SNVs and 88.7% for indels.

Conclusion:

The reproducibility, agreement with reference method and technical positive predictive values were very good for SNVs and acceptable for indels calling (indels lead to more mismatches with capture probes than SNVs). Thus, the test method is suitable for clinical sequencing.

B-134

Identification of microRNAs as Predictive and Supervisory Biomarkers for SCA/MJD Progression

S. Guo, M. Wang, Z. Meng, Y. Zhou, L. Jiao, B. Ying. *West China Hospital, Sichuan University, Chengdu, China*

Backgrounds: Spinocerebellar ataxia type 3/Machado-Joseph disease (SCA3/MJD) is the most common autosomal dominant spinocerebellar ataxia and one of many inherited polyglutamine (polyQ) neurodegenerative diseases. Nevertheless, the exact mechanism of SCA3 is unclear and it still remains an untreatable disorder. At present, microRNAs (miRNAs) have been attracting extensive research interest in different human diseases, which emerged as key regulators via different biological functions in genetic and epigenetic processes, but it remains largely unknown if they are correlated with SCA3/MJD progression. Therefore, the objective of this work was to investigate the potential role of miRNAs in SCA3/MJD development.

Methods: We adopted next-generation sequencing(NGS)to examine the expression profile of miRNAs in cerebrospinal fluid(CSF)samples and peripheral blood samples from 11 SCA3/MJD patients and 10 healthy controls. In order to elucidate potential functions and signaling pathways involved in the pathogenesis of SCA3/MJD, we applied Gene ontology(GO) and Kyoto Encyclopedia of Genes and Genomes(KEGG) pathway analysis to select potential candidate miRNAs for further validation. Quantitative real-time reverse transcription polymerase chain reaction(qRT-PCR) was performed to validate NGS data. ROC analysis was also used to evaluate the predictive power of candidate miRNAs. The qRT-PCR results combined with medical records were further analyzed statistically attempting to reveal the potential relationship between miRNAs expression levels and disease characteristics including disease onset, disease history, etc.

Results: Our results showed that miRNAs profiles presented a total of 198 miRNAs commonly expressed in both CSF samples and peripheral blood samples of SCA/MJD patients. Among them, 14 miRNAs were upregulated and 42 miRNAs were down-regulated in SCA3/MJD group. After functional analysis, 10 differentially expressed miRNAs were chosen for validation among which the expression level changes of 5 miRNAs estimated by qRT-PCR were in accord with NGS data. With the extension of disease onset, the expression levels of three candidate miRNAs including hsa-miR-608, hsa-miR-4739 and hsa-miR-1254 at different stages of the clinical course presents an obvious up-regulated trend, as the miRNAs expression were lower in patients with shorter disease history while overexpression of three miRNAs were found in those who are suffering at an advanced stage, indicating the close positive correlation of miRNAs and disease progression.

Conclusion: These findings were the first report of association between differentially expressed miRNAs and SCA3/MJD development, indicating a possible role for miRNAs as predictive and dynamic supervisory biomarkers of disease progression. Also, our results provided novel insights into

the mechanisms of the pathological process as well as important cues for further functional studies of the disease.

B-135

Intronic Variant in SPTB Gene as Suspected Cause of Hereditary Spherocytosis in a Brazilian Family: Segregation in the Family and RNA Analysis

C. Nobre, T. Santa Rita, P. Mesquita, R. Jácomo, L. Nery, G. Barra. *Sabin Medicina Diagnóstica, Brasília, Brazil*

Background:

Hereditary spherocytosis (HS) is a hereditary hemolytic anemia that may present with an autosomal dominant or non-dominant inheritance pattern. The major genes related to this pathology are SPTA1, SPTB, ANK1, SLC4A1, and EPB42. In a previous study, we analyzed the coding regions ±10bp flanking intronic sequences of these genes in 18 patients diagnosed with HS. Pathogenic or likely pathogenic mutations that explain the condition could be found in 16 patients, except in two of siblings. In this study, we amplified the analysis to coding regions ±50bp flanking intronic sequences and tested other family members (affected and non-affected) in order to identify the variant associated with HS in this family.

Methods:

The DNA was extracted from the EDTA-whole blood using MagNA Pure 24 (Roche). DNA and sequencing libraries were qualified/quantified using TapeStation4200 (Agilent). Coding regions ±50bp flanking intronic sequences of the exome enriched using SureSelect Human All-Exon-V5 Exome (Agilent), SureSelectXT Target Enrichment System (Agilent) and sequenced (2x150-bp High Output V2 Reagent) using NextSeq-500 (estimated mean coverage-150X). Read alignment and variant calling were performed with BWA enrichment (BaseSpace Sequence Hub - Illumina). Variant annotation and filtration were performed with Variant Interpreter (Illumina). Hereditary spherocytosis sub-panel was analyzed (SPTA1, SPTB, ANK1, SLC4A1, and EPB42). SNVs and small indels (<5bp) with total-read-depth >20X, variant-allele-depth >8X and variant-read-frequency >20% were analyzed. Some family members were tested by ARMS-qPCR for a suspected intronic variant: 1 sibling (healthy), the father (healthy), 4 aunts (healthy), and 2 children (affected with HS)]. The variant effect over SPTB intron 28 splicing was studied on whole blood total RNA by RT-qPCR and agarose gel electrophoresis. Approval in the research ethics committee CAAE: 51112215.0.0000.0023.

Results:

A rare variant (c.6023-30G>A) located in the intron 28 of the SPTB gene that co-segregates with HS phenotype in the family was detected in the two siblings and in their two children (all heterozygotes). The father, aunts, and the healthy sibling did not have the variant (all unaffected). This variant was not found in any public population database. To prove the association of the c.6023-30G>A with HS we analyzed SPTB intron 28 splicing by RT-qPCR and no splicing defect or alteration could be detected.

Conclusion:

Here we showed that a rare variant c.6023-30G>A on intron 28 of the SPTB gene co-segregates with the HS phenotype in a family of 4 affected and 6 unaffected individuals. No coding regions variant was observed in the HS genes. RNA analysis could not detect any effect of this variant over SPTB intron 28 splicing. However, we cannot exclude the degradation of the altered transcript by the nonsense-mediated mRNA decay nor if the variant just co-segregates with the real causal variant. Further mRNA and red blood cells membrane protein analysis are in progress.

B-136

Cross Priming Amplification for Rapid Detection of Acinetobacter Baumannii and blaOXA-23 Carbapenemase Gene

H. Lai[1], C. Yan[1], Y. Xie[2]. [1]*West China School of Medicine, Sichuan University, Chengdu, China,* [2]*Laboratory of Clinical Microbiology, Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, China*

Background: Acinetobacter baumannii is a significant opportunistic and multidrug-resistant pathogen responsible for nosocomial infections. Carbapenemase genes, such as blaOXA-23, are responsible for acquired resistance of A. baumannii to carbapenems. Accurate and rapid identification of drug resistance is essential for antibiotic treatment. Here, we describe an isothermal amplification assay for A. baumannii infection screening and drug-resistance gene detection. Cross priming amplification (CPA) is a novel technique for molecular detection based on isothermal amplification reactions, which is carried out by a strand displacement DNA polymerase (Bst DNA polymerase). Targeted DNA sequences can be amplified at a constant temperature utilizing multiple specific primers (1 or 2 cross primers , 2 displacement primers, 2 detection primers) within 2 hours. In the CPA assay, the 5' end of the cross primer is not complementary to the template, and will be displaced when DNA polymerase extends the upstream displacement primers. Detection primers are labeled with FITC and biotin respectively, which allows the amplicon with labels can be detected using a colloidal-gold strip. The amplified products of CPA can be assayed by 1.5% agarose gel electrophoresis and colloidal-gold strip test. The CPA assay is a sensitive, accurate, rapid, and affordable diagnostic tool that will work in resource-limited settings.

Methods: Bacterial DNA template was extracted from clinical samples and identified by Laboratory of Clinical Microbiology, West China Hospital. A set of CPA-specific primers was designed based on the sequence of blaOXA-23, including 1 cross primer, 2 displacement primers and 2 detection primers. Totally, 11 combinations of different primer concentrations were tested for the optimal primer concentration. The amplification temperature and incubation time were also optimized. This was achieved by setting amplification temperatures at 48, 50, 52, 54 ℃, and incubation time at 30, 40, 50, 60, 70, 80 min, respectively. The sensitivity was determined by analyzing products from a series of tenfold diluted concentrations of blaOXA-23 template ranging from 10^5 to 10^{-2} copies/μl. The specificity was tested in panel of five different bacterial strains.

Results: The CPA assay is capable of rapidly detecting A. Baumannii and blaOXA-23 carbapenemase gene. CPA reaction was carried out in a total 20-μl reaction mixture containing 0.25 mM each of primer F3, B3 and CPR, 0.35 mM of primer DF5B, 0.4 mM of primer DF5F, 0.4 mM of dNTPs, 2μl 10× Bst buffer, 8 units of Bst DNA polymerase large fragment, 3 mM of $MgSO_4$, 0.25M of betaine, and 40ng of template. The CPA reaction was carried out at 52 ℃ for 70 min and stored at 4 ℃.

Conclusion: This study demonstrated that the assay method of CPA was specific and sensitive for the rapid detection of A. Baumannii and blaOXA-23 carbapenemase gene. This assay has potential to be used in developing countries and other resource-limited settings.

B-137

Quantitative Impact of Including or Excluding Synonymous Mutations from Tumor Mutational Burden Utilized as a Pan-Cancer Prognostic Marker

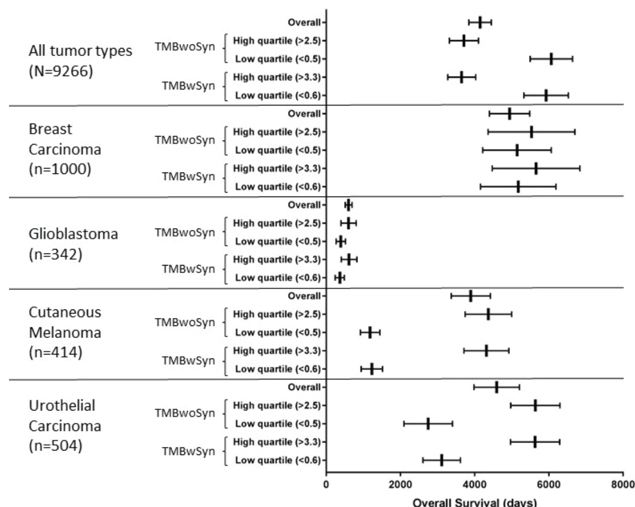
N. Bevins, S. Sun, J. A. Thorson, S. S. Murray. *University of California at San Diego, San Diego, CA*

Background: Tumor Mutational Burden (TMB) is currently reported as part of many molecular tumor profiling methods. Clinicians may utilize TMB to inform prognostication and response to immunotherapy. Standardized methods for TMB calculation are not established, thus, each lab varies in methods with unclear impact on final TMB readout with some labs including synonymous variants whereas others exclude them. We seek to quantify the impact of including synonymous variants on TMB determinations.

Methods: We utilized the published tumor profiles and associated clinical outcomes data of 9266 tumors of various tissue origins with associated outcomes data from The Cancer Genome Atlas. TMB was calculated from whole exome variant calls with or without inclusion of synonymous variants. A denominator of 30 Mb was used to derive the TMB value in units of # of mutations/ Mbase. Statistical analysis was performed using SPSS (IBM).

Results: Mean TMB including both synonymous and non-synonymous variants (TMBwSyn) was 4.90 (range 0.03 - 464.7). Mean TMB excluding synonymous variants (TMBwithoutSyn) was 3.65 (range 0 - 347.7). The difference in means between the TMBwSyn and TMBwithoutSyn was significant using a paired t-test ($p=1.5x10^{-7}$). The mean ratio of TMBwSyn/TMBwithoutSyn was 1.35 (range 1.0 - 6.0, Standard Deviation= 0.22). Comparison of Overall Survival segmented by quartiles of TMBwSyn and TMBwithoutSyn for the overall data set and subsets of tumor types are shown in the figure below. Findings differed by tumor type and clinical endpoint (additional data not shown).

Conclusion: Our results indicate that TMB with inclusion of synonymous mutations retains the prognostic utility of TMB without synonymous mutations if cut points are adjusted appropriately. Identification of prognostic TMB cut points will require tumor type specific analysis.



B-138

Pharmacogenomics and CYP2D6 Copy Number Variation Testing Using Automation Improves Turnaround Time and Lowers Repeat Rate

E. Briggs[1], W. Wooderchak-Donahue[1], R. Melis[1], Y. Ha[1], H. Pham[1], C. Laub[1], A. Roberts[1], G. A. McMillin[2]. [1]*ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT,* [2]*Department of Pathology, University of Utah, Salt Lake City, UT*

Background: Cytochrome P450 2D6 (CYPD26) is involved in the metabolism of approximately 25% of prescription drugs. CYP2D6 has many genetic polymorphisms that can affect enzyme activity and expression. In addition, about 12.6% of people in the US have a *CYP2D6* copy number variation (CNV) that can alter the phenotype prediction. Because of this, *CYP2D6* is found in pharmacogenomics (PGx) panels designed to guide drug and dose selection decisions. Here, we describe the automation of 1) an OpenArray genotyping panel designed to target 120 PGx variants from 36 genes including *CYP2D6*, and 2) a complementary *CYP2D6* CNV assay, each performed with the QuantStudio 12K Flex instrument (ThermoFisher Scientific).

Methods: Using the Tecan Fluent 1080, DNA is normalized to 50 ng/µL. Next, 3 µL DNA is added to 3 µL of OpenArray Genotyping master mix in a 384 well plate. An OpenArray AutoLoader transfers samples from the 384 well plate onto the PGx OpenArray chip.

For the CNV assay, two master mixes are prepared with probes that specifically interrogate *CYP2D6* exon 9 and intron 6 (ThermoFisher Scientific). Each master mix is loaded onto the Tecan along with a DNA plate normalized to 5 ng/µL, and 4 controls. Then, 16µL of each master mix is dispensed into two respective plates. Normalized DNA (4µL) for each sample is added in quadruplicate to both plates. Both genotyping and CNV assays are performed using the QuantStudio instrument.

The accuracy of the automated PGx OpenArray assay was evaluated using 71 DNA samples with known genotypes. Six DNA samples (2 from whole blood and 4 from saliva) were used to assess between and within run precision. Genotypes were assigned for all 120 variants per sample using TaqMan Genotyper software.

Accuracy for the CNV automated assay was evaluated using 60 samples previously characterized with a manual method. Five samples were evaluated within and between runs to test precision. Data was analyzed using CopyCaller software. To assess the reproducibility of the four technical replicates per sample, the standard deviation should be less than 0.05, indicating optimal technical replicate reproducibility, and any sample with a standard deviation >0.09 is suspect.

Hands-on time and repeat rates for manual versus automated testing on both assays was compared.

Results: All 120 genotyping assays performed reproducibly the genotypes among all precision runs were 100% concordant. No false negative results were observed and analytical specificity of 99.9% was obtained. Importantly, DNA extracted from both whole blood and saliva yielded high quality genotype results.

The CNV results were 100% concordant when compared to results obtained previously. Only two samples had a technical replicate standard deviations >0.09, both of which were correctly called as having 3 or more copies.

When compared to manual, hands on time was reduced using automation by 2 hours per 46 sample run, and failure rates lowered from ≤5% to ≤2%.

Conclusion: Our results demonstrate accurate, robust performance of the automated PGx OpenArray and CNV assays. These updates have reduced repeat rates and improved turnaround time.

Wednesday, August 7, 2019

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

Nutrition

B-140

Comparison of Serum Calcium Level in Normotensive and Hypertensive Pregnant Women

C. F. Chukwunyere. *Federal Medical Centre Abeokuta, Abeokuta, Ogun, Nigeria*

Background: Deficiency of calcium among pregnant women has been linked to the possibilities of development of hypertensive disorders of pregnancy. Identification of possible risk factors and baseline calcium level would help in prevention of hypertensive disorders of pregnancy which is presently the leading cause of maternal mortality in our country.

Aim: To compare maternal serum calcium level of women with hypertensive disorders of pregnancy (gestational hypertension and pre-eclampsia) and normotensive pregnant women.

Methods: This was a descriptive study among patients diagnosed with hypertensive disorders of pregnancy at the Federal Medical Centre, Abeokuta during the study period, while the comparative group was normotensive pregnant women with at least two normal blood pressure reading and no history of chronic hypertension or proteinuria. Case to comparative group was 1:1. Frequency matching was conducted in terms of age, parity, body mass index and dietary habits.

Results: This study found that the mean age of normotensive control was 31.89±4.97yrs,women with gestational hypertension (GH) was 31.14±3.24 yrs and preeclampsia (PE) was 31.89±5.23 yrs. The mean serum calcium level in normotensive controls was 2.64±1.38 mmol/l, women withGH was 2.39±1.15mmol/l and PE was 2.08±0.76mmol/l(p=0.049). The incidence of hypocalcaemia was 33% in normotensive controls, 51.1% in GH and 51.1% in PE. There was inverse relationship between milk consumption and gestational hypertension (p=0.003) and preeclampsia (p=0.036).

Conclusion: This study revealed that significant association exists between mean serum calcium level in gestational hypertension and preeclampsia. This study also found that dietary intake of milk could lower the risk of gestational hypertension and preeclampsia.

Recommendation: The hypocalcaemia in our environment was quite enormous, emphasis on calcium supplementation both through drug therapy and dietary approach can help in reducing the burden of hypertensive disorders of pregnancy within our environment.

B-141

Iodine Status of Euthyroid Adults: A Cross-Sectional, Multicenter Study

D. Wang, S. Yu, Y. Yin, S. Xie, Q. Cheng, H. Li, X. Cheng, L. Qiu. *Peking Union Medical College Hospital, Chinese Academic Medical Science and Peking Union Medical Col, Beijing, China*

Background: Iodine, an essential nutrient, is the most important trace element in thyroid hormone synthesis and maintenance of thyroid function. This study investigated the iodine nutrition status in healthy Chinese adults and assessed the relationship between urinary iodine concentration (UIC) and thyroid hormone levels.

Methods: A cross-sectional, multicenter study was conducted between October 2017 and January 2018, with 1017 adults recruited from five cities in China. All subjects underwent thyroid ultrasonography and only those with normal results were included in the study. UICs were measured by inductively coupled plasma mass spectroscopy, and adjusted using urine creatine levels. Thyroid hormone levels were measured using an automated immunoassay analyzer.

Results: The median UIC and adjusted UIC were 134.0 µg/L and 114.2 µg/g, respectively. UIC was not significantly different between males and females (*P*=0.737). However, the adjusted UIC was significantly different between sexes (P<0.001). The median UIC was higher than 100 µg/L. According to the World Health Organization criterion (100 µg/L), the total prevalence of iodine deficiency is 33.1% (n=271). The prevalence rates of iodine deficiency in our study were 33.2% and 32.9% in males and females, respectively, and had no difference between sexes and among cities (P>0.05). Serum thyroid-stimulating hormone (TSH) levels increased when UIC increased. The Kruskal-Wallis test showed no significant differences in free triiodothyronine, free thyroxine, and TSH, with different levels of UIC (all P >0.05).

Conclusion: Chinese adults with normal thyroid structure have relatively sufficient iodine levels.

B-142

Quantitative Fecal Fat Analysis by NMR Spectroscopy

J. Lu[1], R. A. Jensen[2], J. O. Bird[2], L. M. Johnson[3]. [1]*ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT,* [2]*ARUP Laboratories, Salt Lake City, UT,* [3]*Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT*

Background: Measurement of fecal fat can be useful for patients with maldigestion and malabsorption due to pancreatic or intestinal diseases. Nuclear Magnetic Resonance (NMR) spectroscopy is a simple method that laboratories use to accurately quantitate the percent fat in stool and then calculate the average grams/day of fat excreted in children and adults. **Objective:** To describe the validation of the CEM SMART Trac™ II Fat Analysis System (CEM Corporation, Matthews, NC) that combines pre-analytical weighing and microwave drying with analytical analysis of the percent fat in stool samples using NMR spectroscopy. **Methods:** The total stool weight was recorded with the duration of collection (24, 48, 72 hours) prior to processing the specimen. A small amount (1.75-2.75 grams) of a well-homogenized sample was weighed and dried by microwave to remove water interferences. The sample was then analyzed by NMR spectroscopy to determine the percent fat. The NMR signal is the free induction decay (FID). FID of lipid protons occurs at a slower rate than that of protons from other substances in the stool. The percent fat was converted to grams fat/24 hours based on the total weight of the specimen and the collection duration. Method accuracy, linearity, imprecision and sensitivity were determined. **Results:** To assess accuracy, the percent fat of residual patient fecal samples (n=35) were determined on the SMART Trac II system and compared to the SMART Trac I system. The samples spanned the measurement range between 1-30% fat. Deming regression produced a slope of 0.95 and y-intercept of -0.05 (R^2=0.975). Samples for linearity experiments were created by mixing a high fat sample with a low fat sample into a set of 5 different ratios that spanned 0.6-29.3% fat and tested in duplicate. Linear regression generated a slope of 0.966, y-intercept of 0.0 (R^2=1.00). Precision was verified by analyzing two quality control samples in triplicate each day for 5 days. Within-run and total imprecision were 4.5% CV and 5.2% CV at 1.42% fat and 2.4% CV and 2.6% CV at 11.6% fat, respectively. A no fat control sample was analyzed in 10 replicates, and the mean of 0.53% fat and standard deviation of 0.018% fat were used to find the limit of blank at 0.59% fat (LOB = $Mean_{blank}$ + $3SD_{blank}$). The low quality control sample of 1.42% fat was analyzed in 10 replicates, and the standard deviation of 0.066% fat was used to calculate the limit of detection at 0.79% fat (LOD = LOB + $3SD_{lowQC}$). We analyzed the distribution of percent fat measurements from both adults and children samples from the past year. The distributions were right-skewed, reflecting that some patient samples have very high percent fat measurements. The ranges of the adult (6-96 years old) and children (0-5 years old) samples were 0.5-23.3% fat and 1.1-21.5% fat with medians of 2.7% fat and 3.5% fat, respectively. **Conclusions:** The fat content of a stool samples can be accurately and precisely measured with the CEM SMART Trac II Fat Analysis System.

B-143

Alterations in Renal Cortical Mitochondrial Dynamics and Mitophagy during the Normoalbuminuric Stage of Diabetes Mellitus

N. Ishii[1], P. K. Carmines[2], A. Imoto[1], Y. Kurosaki[1], H. Ikenaga[3], M. Yokoba[1], T. Ichikawa[1], T. Takenaka[4], M. Katagiri[1]. [1]*Kitasato University School of Allied Health Sciences, Sagamihara, Japan,* [2]*University of Nebraska Medical Center, Omaha, NE,* [3]*Seiikai, Tochigi, Japan,* [4]*International University of Health and Welfare School of Medicine, Tokyo, Japan*

Background: Oxidative stress during the normoalbuminuric stage of type 1 diabetes mellitus (DM) damages renal cortical mitochondria (Clin Sci 124:543-52, 2013). Damaged mitochondria must be disintegrated and removed to prevent their accumulation and subsequent renal dysfunction.

Objective: We aimed to determine if oxidative stress in DM triggers renal cortical mitochondrial dynamics (fission and/or-, fusion) and elimination through mitochondria-selective autophagy (mitophagy).

Methods: Four groups of rats were studied: 1) STZ group (*n*=6): rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg, *i.p.*), 2) Sham group (*n*=6): rats receiving the STZ vehicle, 3) STZ+TLM group (*n*=5): STZ rats treated with telmisartan (TLM, an angiotensin receptor blocker; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group (*n*=5): TLM-treated Sham rats.

Two weeks later, blood glucose concentration, blood pressure, glomerular filtration rate, and urinary excretion of albumin and *N*-acetyl-β-D-glucosaminidase (NAG) were measured in each rat.

The following parameters were quantified in renal cortex: 3-nitrotyrosine (3-NT) production (an oxidative stress marker) by HPLC, expression levels of fission (Drp1)-, fusion (Mfn2)-, and mitophagy (PINK1, BNIP3, LC3-II, p62)-related proteins by Western blot, and localization of these proteins by immunohistochemistry.

Results: Blood glucose levels were higher in STZ rats than in Sham rats (P<0.05) and were unaffected by TLM. Blood pressure, urinary albumin excretion, and NAG excretion were not altered by STZ, nor by TLM treatment. Compared with Sham rats, glomerular filtration rate and renal cortical 3-NT production were increased in STZ rats (P<0.05), and both changes were prevented by TLM (P<0.05).

Renal cortical Drp1 levels were 3-fold higher in STZ than in Sham rats, with STZ+TLM rats exhibiting intermediate levels of this fission marker. In contrast, levels of the fusion marker Mfn2, did not differ among groups.

STZ rats displayed increased renal cortical LC3-II and PINK1 protein expression compared with Sham rats (P<0.05), with these effects prevented by TLM (P<0.05). BNIP3 (dimer) and p62 levels did not differ among groups. Immunohistochemical staining confirmed that the mitophagy-related proteins in STZ were located at renal tubular sites.

Conclusions: These data suggest that fission, but not fusion, of renal cortical damaged mitochondria is enhanced by in DM. TLM-sensitive accumulation of PINK 1 and LC3-II the tubular epithelium during DM suggests that oxidative stress activates the PINK1-parkin pathway, in which PINK1 binding to LC3-II (an established marker for autophagosome formation) results in mitochondrial elimination via autophagy.

B-144

Decreased Endogenous ω-6 PUFAs Induced Intestinal Mucosa Transcriptional Reprogramming that Contributed to Amelioration of Intestinal and Liver Injury in Mice in a Context of Systemic Inflammation and Chronic Ethanol Exposure

D. Warner, J. Warner, Y. Song, C. McClain, I. Kirpich. *University of Louisville, Louisville, KY*

Background/Objectives: Gut-derived bacterial lipopolysaccharides (LPS) play an essential role in inducing intestinal and systemic inflammatory responses and have been implicated as a pathogenic factors of ethanol (EtOH)-associated multi-organ pathology, including the gut and liver. Previous work from our group demonstrated deleterious effects of a diet with high levels of ω-6 polyunsaturated fatty acids (PUFAs) on the gut-liver axis in mice chronically fed EtOH, specifically, elevated gut permeability, as well as intestinal and liver injury and inflammation. The goal of the present study was to investigate the effects of a decrease in endogenous ω-6 PUFAs on a crosstalk between the intestine and liver in a mouse model of systemic inflammation in a setting of chronic EtOH administration. **Methods:** Wild type (WT) *and fat1* transgenic mice (that endogenously convert ω-6 to ω-3 PUFAs resulting in decreased ω-6 PUFA levels) were placed on control or EtOH-containing diets for 6 weeks. To induce systemic inflammation, a single LPS dose (5g/kg) was given 24h prior to euthanasia. Liver injury was determined by plasma ALT activity and histological assessment of steatosis, neutrophil infiltration, and apoptosis. Intestinal pathological changes were evaluated by histology. RNAseq analysis of ileum was performed to identify differentially expressed genes between experimental groups. Fecal bile acid analysis was performed by HPLC. Expression of hepatic genes involved in bile acid metabolism were evaluated by qRT-PCR. **Results:** Ethanol-fed, LPS-challenged *fat1* mice developed milder liver injury compared to WT littermates (ALT 73±16 vs. 400±95 U/L, P <0.05). *Fat1* mice also had less hepatic neutrophil infiltration and apoptosis following EtOH+LPS treatment. Histological analysis of intestinal tissue revealed alterations in ileum morphology in response to EtOH and LPS, including epithelial damage with superficial necrosis of villi that were more pronounced in WT vs. *fat1* mice. Intestinal inflammation was significantly higher in EtOH+LPS treated WT mice when compared to *fat1* treated mice, and characterized by enhanced numbers of neutrophils and mononuclear cells, and accompanied by increased gene expression of the pro-inflammatory cytokine *Il6*, and neutrophil chemoattractants *Cxcl1 and Cxcl2.* RNAseq analysis revealed significant intestinal mucosa transcriptional changes in WT vs *fat1* mice in response to chronic EtOH and LPS exposure, including pathways related to inflammation and antimicrobial defense. Expression of numerous members of the cytochrome P450 and sulfotransferase families, important xenobiotic/bile acid-metabolizing enzymes, were down-regulated in WT vs. *fat1* EtOH+LPS treated mice, including *Cyp3a11* and *Cyp2b10* (45- and 100-fold, respectively). Further, expression of genes responsible for bile acid metabolism in the liver were also down-regulated in WT vs. *fat1* EtOH+LPS treated mice. The compromised ability of the intestinal mucosa and the liver to metabolize/detoxify bile acids was associated with increased levels of the lithocholic acid, a hepatotoxic secondary bile acid. **Conclusions:** Our findings demonstrate that lowering ω-6 PUFAs attenuated EtOH+LPS-induced pathology of the intestine and liver, potentially through preservation of the xenobiotic/bile acid detoxification system and thereby preventing adverse effects of hepatotoxic bile acids in the liver.

B-145

Quantitative Analysis of Long Chain Fatty Acids (C12-C24) in Packed Red Blood Cells by Gas Chromatography-Mass Spectrometry

B. Lozier[1], N. Phengphong[2], M. Manion[2], E. Schwarz[1], E. Kish-Trier[1], J. Straseski[3], I. De Biase[3], T. Yuzyuk[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]University of Utah; ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT*

Background: As a major component of lipids, long-chain fatty acids (LCFAs) play important roles in a variety of physiological processes such as energy storage, inflammatory response, membrane function and signal transduction. Patients with malabsorptive disorders and those receiving prolonged parenteral nutrition are at risk of developing essential fatty acid deficiency; hence, circulating LCFA levels are closely monitored in these individuals. Due to much lower intra-individual variability of LCFAs, red blood cells (RBCs) is considered the preferred sample type over plasma or serum for evaluating long-term nutritional status. Here, we present the clinical validation of a gas chromatography negative chemical ionization-mass spectrometry (GC-NCI-MS) method for the quantitation of total levels of 22 LCFAs in packed RBCs, including essential omega-3 and omega-6 fatty acids.

Methods: RBCs were washed with normal saline and lysed by freezing. Following acid/base hydrolysis, samples were derivatized with pentafluorobenzyl bromide and analyzed by GC-NCI-MS (Agilent 5977A/7890B). Analyte responses were normalized to 12 isotopically labeled internal standards. LCFA concentrations were calculated using calibration standards included in every run. Analysis was performed with Agilent MassHunter software. Whole blood from 6 donors was used for determination of sample stability and appropriate handling conditions. Reference intervals were established testing >200 samples from adult and pediatric controls (0-61 years of age) using EP Evaluator (Data Innovations). Samples from 20 donors collected after overnight fasting and 4-5 hours after a meal were also tested to assess the effect of dietary intake on RBC LCFA quantitation.

Results: The method was linear (r^2>0.99) across the analytical measurement range for each LCFA. The accuracy in calibrators was within 70-130% of target at the low limit of quantitation and 85-115% at all other levels, with a coefficient of variation ≤20% (n=16). Intra-assay (n=6 on 2 runs) and inter-assay precision (n=16) assessed in donor RBC lysates showed variability <10% for values >10 nmol/mL and <16% for values ≤10 nmol/mL. Analytes in unprocessed whole blood were found to be stable for at least 24 hours at room temperature (RT), and 5 days refrigerated (2 - 8°C) after draw. LCFAs in lysed RBCs were stable for 24 hours at RT, and at least 9 months at ≤-65°C. EDTA, heparin, ACD and PPACK (Phe-Pro-Arg-chloromethylketone) tubes were all suitable for blood collection. Importantly, the difference between fasting and postprandial RBC LCFA concentrations was comparable with intra-assay precision of the method (<10%), unlike plasma (>30% for some LCFAs). Results from adult and pediatric controls, expressed in absolute concentrations or as percent of total LCFAs, were used to establish age specific reference ranges.

Conclusion: The validated GC-NCI-MS method quantifying 22 LCFAs in packed RBCs can be used for the evaluation of long-term nutritional status in a clinical setting. RBC results are unaffected by daily diet, eliminating the need for fasting, which is particularly difficult for pediatric patients or patients prone to metabolic decompensation. Established age specific ranges will help with clinical interpretation of laboratory results in different patient populations.

B-146

HLA-tagging SNP may not be Suitable for DQ2.5 Risk Prediction for Brazilian Celiac Population

M. A. Pereira[1], L. R. Leite[2], I. B. R. Paula[3], B. D. C. V. Cota[3], A. A. Morais[2], N. L. Viana[2], F. S. V. Malta[1], R. L. M. Guedes[1], L. B. Alvim[3], M. M. Freire[2], D. Zauli[1]. *[1]Hermes Pardini Institute (Research & Development Division), Belo Horizonte, Brazil, [2]Hermes Pardini Institute (Progenética Laboratory), Belo Horizonte, Brazil, [3]Hermes Pardini Institute (Molecular Genetics Division), Belo Horizonte, Brazil*

Background: Celiac disease (CD) is a gluten-sensitive autoimmune disorder, estimated to affect 1:100 people around the world. In Brazil, there are no epidemiological studies available to confirm this estimative but a similar proportion is expected due to historical miscegenation. CD results from the combination of genetic (human leukocyte antigen: HLA) and environmental (gluten) factors. HLA class II genes are strongly associated with CD predisposition, especially the DQ2.5 (DQA1*05/DQB1*02) and DQ8.1 (DQA1*03/DQB1*0302) haplotypes. Almost 95% of CD patients carry at least one of the two risk molecules (DQ2.5 or DQ8.1); however, up to 40% of healthy people also carry one of these haplotypes, so their presence alone is not diagnostic of CD. Testing for HLA risk molecules is routinely performed by different methods, e.g. Tagging SNP, PCR-SSOP and MLPA. In our lab, an in-house assay and HLA-tagging SNP (Monsuur *et al,* 2008) are commonly used for CD risk detection. The usage of six tagging SNPs has proven high specificity and sensitivity (>95%) in European celiac populations (Finnish, Hungarian and Italian). However, there are no studies using Brazilian population or comparing these methods. **Objective:** This report compares HLA-Tagging SNP and our in-house assay in order to assess the consensus between both methodologies as predictive tools for DQ2.5 and DQ8 presence. **Methodology:** Genomic DNA was extracted from 168 blood or buccal swab samples. The HLA-tagging SNP was performed as Monsuur *et al*. (2008). The in-house assay consists of DQ2.5 (DQA1*0501/ DQB1*0201) and DQ8 (DQB1*0302) detection, through allele-specific PCR and fragment analysis followed by AmbiSolv® (Thermo Fisher), respectively. **Results and Discussion:** For DQ8 (n=168), 161 (95.8%) samples presented concordant results between both methodologies while the 7 remaining (4.2%) presented inconclusive AmbiSolv results. For DQ2.5, 32 samples failed for one of both methodologies and were discarded. For the remaining (n=136), 34 (25%) samples presented discordant results with DQ2.5 positive only for the in-house assay. **Conclusions and Perspectives:** The demand for CD genetic tests has increased in the past few years, and reliable determination CD genetic predisposition can avoid unnecessary biopsy and gluten-free diet prescriptions. HLA-tagging SNP is a cost-effective population screening method for CD considered as highly accurate, but it has been only validated in European populations. Our results are a strong indicative that this technique may not be suitable for DQ2.5 analysis on Brazilian population. To solve this inconsistency, a pilot case-control study with 20 CD positive and 20 CD negative patients will be performed. Random patients and inconclusive results will also be analyzed by PCR-SSOP to broaden HLA-typing and confirm the CD risk correlations between methodologies.

B-147

Association Histopathological Prognosis with the Serum Concentration of Vitamins A <&> E in Papillary Thyroid Carcinoma

L. C. L. Silva[1], T. C. T. de Melo[1], D. M. C. Santos[1], J. N. G. de Araújo[1], A. G. C. L. Silva[1], K. D. S. R. Rodrigues[1], S. R. M. H. Tarrapp[2], C. C. O. Ramos[2], V. N. Silbiger[1]. *[1]Universidade Federal do Rio Grande do Norte, Natal, Brazil, [2]Hospital Liga Norte Riograndense Contra o Câncer, Natal, Brazil*

Background: Thyroid cancer (TC) is the most common malignancy of the endocrine system. According to last estimates from the Brazilian National Cancer Institute (INCA), this neoplasm is expected to reach 9610 people in Brazil by the end of 2018, and the largest number of cases is expected to occur in the Northeast region. Papillary thyroid carcinoma (PTC) accounts for about 70% of TC cases and considering that the rate of incidence varies in different parts of the world, modifiable risk factors may play an important role in TC carcinogenesis. Experimental and clinical studies have shown that antioxidant vitamins can inhibit the development and progression of cancer, thus the biochemical status of vitamins A and E have emerged as potential markers of risk for genesis and evolution of the disease. Therefore, the present study aimed to investigate the serum concentrations of vitamins A (retinol, ROH) and E (α-tocoferol, TOH) with histopathological prognostic factors of CPT.

Methods: Serum vitamin A and E were measured in three times using liquid chromatography: one before thyroidectomy and two after thyroidectomy of 46 PTC patients.

Results: Reduced serum ROH concentrations were associated with the presence of extrathyroidal extension (OR: 11.733, 95% CI: 1.326-103.795, p = 0.027) and lymph node metastases (OR: 4.94, 95% CI: 1.367-17.049, p = 0.015). Likewise, lower concentrations of TOH 60 days after surgery were associated with the presence of angiolymphatic invasion (OR: 9,800; 95% CI: 1,089-88,229; p = 0,042). We suggest that both food intake and vitamin A status and may be relevant in predicting histopathological outcomes of CPT.

Conclusion: Our findings suggest that higher serum ROH and TOH concentrations in patients with PTC may be associated with a lower predisposition to develop tumors with less aggressive histopathological outcomes and thus, regular monitoring of the biochemical status of these vitamins may provide effective subsidies for intervention strategies precocious and nutritional counseling of patients with TC.

B-148

Frequency of Metabolic Syndrome in Community Kitchen Users over 18 Years Old in Lima, Peru

A. Y. Gallo Ramirez[1], J. G. Villena Pineda[1], L. Rodriguez Malqui[2], A. C. Palacios Ramirez[1], G. C. Ferreyros Brandon[1], S. M. Flores Toledo[1], J. C. Jara Aguirre[1]. *[1]Facultad de Medicina, Escuela de Tecnologia Medica. Universidad Peruana Cayetano Heredia, Lima, Peru, [2]Servicio de Laboratorio Clinico de Emergencia, Hospital Nacional Sergio Bernales, Lima, Peru*

Background: Metabolic syndrome (MS) combines factors as hypertension, dyslipidemia, abdominal obesity and glucose intolerance due to insulin resistance, which increases the likelihood of diabetes and cardiovascular disease. Community kitchens were designed as a supplementary alimentary support in order to meet food needs of persons with population of poverty and extreme poverty and receive a support from the Complementary National Program of Food Assistance. An inadequate nutritional program at these community kitchens can trigger a malnutrition or obesity state. The objective of this study was to determine the frequency of metabolic syndrome in diners over 18 years of age who attend community kitchens in the district of San Juan de Miraflores, Lima, Peru.

Methods: An observational, descriptive cross-sectional study was conducted in October of 2018 and 171 regular diners who attended to three community kitchens (San Martin, Niño Jesus, Comite de Damas) at the San Juan de Miraflores district in Lima, Peru were included. Information of age, gender, weight, height, arterial blood pressure and waist circumference were obtained. A fasting whole blood samples were collected using BD Vacutainer Plastic SST gel tube following the CLSI GP41 collection of diagnostic venous blood specimen guideline and the serum samples were collected after centrifugation (1300g for 10 min). Serum glucose, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were performed using ABX Pentra reagents (France) and Pentra c400 HORIBA (Japan) automated chemistry analyzer; calculated LDL cholesterol was performed using the Friedewald equation when triglycerides <400mg/dL. Metabolic syndrome was established using the National Program of Cholesterol Education Panel III of Treatment in Adults (NCEP-ATP III) criteria.

Results: Of 171 regular dinners who attend regularly to these community kitchens, 122 (71%) were females and 49 (29%) males, with an age mean and SD of 55±17 (range 18-84). The overall frequency of metabolic syndrome in the regular attendees was 44% (n=76). The frequency of MS was 28% (n=21), 34% (n=26) and 38% (n=29) at the San Martin, Niño Jesus, Comite de Damas community kitchens, respectively. MS was more frequent in women 54% (n=66) than in men 20% (n=10) (p <0.05). Among the attendees with MS, 59% (n=45) had at least three NCEP-ATP III metabolic syndrome criteria, 32% (n=24) four criteria, and 9% (n=7) five criteria. Of these 76 attendees with MS, 92% (n=70) had abdominal obesity/waist circumference, 88% (n=67) hypolipoproteinemia of HDL cholesterol, 78% (n=59) hypertriglyceridemia, 65% (n=49) arterial hypertension and 28% (n=21) fasting glucose ≥110 mg/dL. Between attendees with MS and without MS, metabolic syndrome was associate with female gender (p<0.05), with obesity/waist circumference, systolic arterial hypertension, hypolipoproteinemia of HDL cholesterol and hypertriglyceridemia (p<0.001).

Conclusion: The frequency of metabolic syndrome in the community kitchens was 44% and more relevant in women, the obesity/waist circumference was the most frequent criteria among the diners who had metabolic syndrome. In addition, metabolic syndrome was associated with obesity/waist circumference, hyperglycemia and hypertriglyceridemia that could be related to the type and amount of intake food that they received at community kitchens.

B-149

Generation of Roche Holotranscobalamin Clinical Decision Points by Transference and Evaluation using Methylmalonic Acid and Total B12

C. M. Trambas[1], K. Liu[1], W. Louey[1], J. Nguyen[1], H. Luu[2], E. Wu[1], B. Leung[1], C. Tan[1], K. Wollbrandt[1], E. Capovilla[1], C. Lynch[1], T. Yen[1], K. A. Sikaris[1]. [1]*Melbourne Pathology, Collingwood, Victoria, Australia,* [2]*Shimadzu, Rowville, Victoria, Australia*

Background: Measurement of holotranscobalamin (holoTC) has proven useful in the assessment of B12 status, particularly in the settings of pregnancy and haptocorrin deficiency. Identification of sub-clinical B12 deficiency remains challenging, however, and an intermediate zone in holoTC clinical decision limits is often implemented to assist in distinguishing probable deficiency from likely sufficiency. Such clinical decision limits have been best established for the Abbott Architect assay, the assay longest in use. Recently, Roche introduced their holoTC assay and we sought to investigate possible clinical decision points to aid in the assessment of B12 status. **Objective**: (i) To establish clinical decision points for the Roche cobas e801 holoTC assay by transference of the Abbott Architect reference intervals (< 23 pmol/L deficient; 23-35 pmol/L intermediate; > 35 pmol/L replete); (ii) To evaluate derived decision points using total B12 and methylmalonic acid (MMA). **Methods**: Routine serum samples presenting for B12 testing over the period August-November 2018 were included the study; we preferentially selected samples in the Architect holoTC range 5-50 pmol/L, but included samples over the entire linear range . Aliquots were frozen until analysis of total B12 (cobas e801), MMA (LC-MS/MS), and holoTC (cobas e801 and Architect). Transference of Architect holoTC reference intervals was applied using Passing & Bablok regression. Classification by these decision points was then evaluated by comparing the distribution of total B12 and MMA with Architect classified results. **Results**: Samples from 562 patients (67% women) were analysed; the median age was 45 and the median eGFR >90. By Passing & Bablok linear regression Cobas_e801 holoTC = 8.927 + 1.017 x Architect holoTC (r = 0.816). Application of the Architect clinical decision limits gave corresponding decision limits for Roche of < 34 pmol/L deficient; 34-45 pmol/L intermediate, > 45 pmol/L replete. Application of these decision limits to the total testing cohort classified 51% of the cohort as deficient, 28% as intermediate and 21% as replete compared to respective Architect rates of 48%, 35% and 16%. Median MMA concentrations (25th, 75th) in each respective holoTC class were for Roche 0.63 µmol/L (0.34, 1.17), 0.39 µmol/L (0.26, 0.64), 0.22 µmol/L (0.16, 0.38) and Architect 0.69 µmol/L (0.36, 1.27), 0.38 µmol/L (0.25, 0.67), 0.21 µmol/L (0.15, 0.31). Median B12 concentrations (25th, 75th) were 116 pmol/L (81, 162), 152 pmol/L (111, 191), 182 pmol/L (151, 224) for Roche and 111 pmol/L (78, 161), 150 pmol/L (117, 186) and 193 pmol/L (159, 224) for Architect. **Conclusions**: Roche holoTC clinical decision points generated by transference are < 34 pmol/L (deficient), 34-45 pmol/L (intermediate) and > 45 pmol/L (replete). Application of these decision points to our cohort gave a similar classification of B12 status, and similar distribution of MMA and total B12 results as application of the Architect holoTC decision points.

B-150

Status of Vitamin B_6 Sufficiency in the US Pediatric and Adult Populations

M. M. Kushnir[1], B. Song[2], J. K. Krantz[3], E. L. Frank[4]. [1]*ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT,* [2]*Absorption Systems, San Diego, CA,* [3]*ARUP Laboratories, Inc.,, Salt Lake City, UT,* [4]*Department of Pathology, University of Utah, Salt Lake City, UT*

Background: Deficiency of vitamin B_6, a cofactor for numerous essential biochemical reactions, has been shown to be associated with coronary heart disease, stroke, neurologic conditions, and higher risk for some cancers. Vitamin B_6 status can be assessed by measuring pyridoxal 5′-phosphate (PLP), the phosphorylated biologically active form of the vitamin, in plasma. The conventional technique is HPLC analysis; newer methods utilize mass spectrometry (LC-MS/MS). Limited information is available on vitamin B_6 status in the general US population.

Methods: We reviewed historic PLP concentration data for consecutively analyzed routine patient samples (n>100,000) submitted from all fifty states for testing by a large clinical diagnostic laboratory. Analyzed samples were from children <18y (3%), adults 18-59y (54%), older adults 60-79y (35%), and elderly adults >79y (8%). Out of the samples, 33% were from males and 67% from females; samples were tested using HPLC with fluorescence (FL) detection (49%) or a newly-developed LC-MS/MS method (51%). The HPLC-FL and LC-MS/MS methods were validated according to CLSI guidelines.

Results: The analytical measurement range for both the HPLC-FL and LC-MS/MS assays was 5-500 nmol/L. Total imprecision of the assays was <10%. The linear regression equation, standard error, and correlation coefficient for between-method comparison (n=163) were $PLP_{LC-MS/MS} = 0.93*PLP_{HPLC-FL} + 2.60$; $S_{y/x} = 5.94$ and r= 0.988. No statistical difference in results was observed for a set of patient samples collected during a continuous 30-day period and analyzed while both methods were in routine use (n=9,753; 51% tested by HPLC-FL and 49% tested using the LC-MS/MS method; p=0.85). The PLP reference interval was 20 - 125 nmol/L. Median (central 95%) PLP concentrations in samples from children, adults, older adults and elderly adults were 58 (12-445), 43 (8-334), 46 (7-356) and 43 (6-354) nmol/L, respectively; the range of observed concentrations (central 99%) was <5-2,347 (<5-497) nmol/L. Median concentrations in samples from males and females were 48 nmol/L and 43 nmol/L, respectively. Out of the entire data set, measured PLP concentrations within the reference interval were found in 63% of the specimens; 20% of samples showed PLP concentrations below the deficiency cutoff value of 20 nmol/L and concentrations exceeded the upper reference limit in 17% of the samples. The lowest rate of vitamin B_6 deficiency was in children (7%) and the highest was in elderly adults (24%). In the pediatric and adult age groups, higher PLP concentrations were measured in samples from males than from females (p<0.001). Lower PLP concentrations were observed in samples collected during winter/spring (December - May) than those collected during summer/fall (June - November) months (p<0.0001).

Conclusions: Overall, we found PLP concentrations outside the reference interval in 37% of samples analyzed, indicating appropriate use of the assay to assess vitamin B_6 deficiency and to monitor supplemental vitamin therapy. PLP concentration was deficient in 20% of samples analyzed. Our data demonstrate between sex, among age groups, and seasonal differences in population PLP concentrations, with lower concentrations observed in women, elderly adults, and during the winter/spring months.

Wednesday, August 7, 2019

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

Pediatric and Maternal Fetal

B-152

Serum sFlt-1: PlGF Ratio in Complicated and Noncomplicated Preeclamptic Cases

V. pant, B. K. Yadav, J. Sharma. *Institute of medicine, kathmandu, Nepal*

Introduction: Women with preeclampsia are at an increased risk for life-threatening obstetric or medical complications. Preeclampsia can lead to intrauterine growth restriction, oligohydramnios, birth as well as higher rates of neonatal intensive care unit admission and length of stay. Nepal maternal mortality and morbidity study 2008/2009 revealed 21% maternal death due to Eclampsia. Various recent studies have revealed that the sFlt-1: PlGF ratio (serum soluble Fms like tyrosine kinase -1: Placental growth factor ratio) in the Preeclamptic women is significantly higher compared to controls.

Objective: The purpose of this study is to follow the cases of Preeclampsia till delivery to correlate the serum sFLT1: PLGF ratio with possible Preeclamptic complications in these follow up cases.

Methodology: Singleton live pregnancy in ≥ 20 years of maternal age with weeks of gestation from 20 till delivery presenting with features of Preeclampsia in Gynae/Obs OPD of Tribhuvan University Teaching Hospital were recruited for this study by purposive sampling method. Cases with multiple gestation, advanced age above 35 years, placental insufficiency and hepatic or renal disease were excluded from the study. Total forty four cases of Preeclampsia were selected according to the criteria defined by the American College of Obstetricians and Gynecologists. Blood pressure, urinary protein, serum sFlt-1, serum PlGF and sFlt-1: PlGF ratio was recorded in each case at the time of presentation. Concentration of sFlt-1 and PlGF were measured with commercially available ELISA kits. The cases were followed up till delivery to observe for the possible maternal and fetal complication. SPSS ver. 17.0 was used to analyze the data.

Result: Twenty-seven (61.36%) cases of preeclampsia developed complication when they were followed until delivery. Eclampsia, persistent thrombocytopenia and elevated liver enzymes were observed as maternal complication of preeclampsia. Likewise, low birth weight, intrauterine fetal death, immaturity, intrauterine growth retardation and birth asphyxia were mostly seen as fetal complication. Low birth weight (LBW) was the commonest complication (20.45%) followed by Intra uterine growth retardation (IUGR) (18.18%).

When the groups based on presence or absence of complication was compared with the mean of sFlt-1: PlGF ratio in each group, significantly higher ratio (35.51 ±8.1 vs. 25.4 ± 8.7) was found in the group with presence of complication which was highly significant (P value 0.001)

Conclusion: Preeclamptic women who develop complication has higher ratio of serum sFlt-1/PlGF compared to those who do not develop complication. Further large prospective studies are required to develop this ratio as a possible marker to predict Preeclamptic complication.

B-153

Noninvasive Prenatal Diagnosis of Fetal Achondroplasia using MALDI-TOF Mass Spectrometry

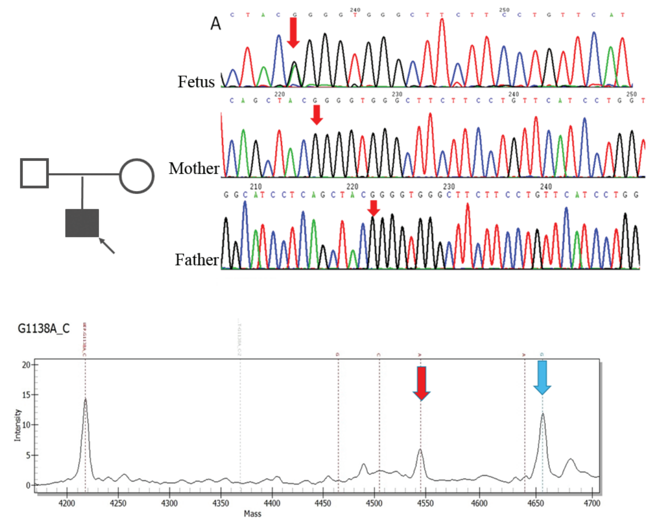
R. Li, J. Duan, Y. Ren, J. Wang, Q. Zhang, Y. Lu, C. Wang. *Chinese PLA General Hospital, Beijing, China*

Background: Achondroplasia (ACH) is the most common form of short-limb dwarfism in humans. The phenotypic characteristics of ACH don't manifest until later in pregnancy, which undoubtedly increase the difficulty of diagnosis. More than 98% of the patients have a *FGFR3* c.1138 G>A mutation, which make genetic diagnosis possible. Our aim is to develop the noninvasive method for early prenatal screening for fetal achondroplasia based on MALDI-TOF Mass Spectrometry.

Methods: Ten cases were referred to the Department of Obstetrics and Gynecology, Chinese PLA general Hospital for suspected skeletal dysplasia from February 2014 to March 2017. Blood samples were collected from each parent. Their *FGFR3* mutations were determined by sanger sequencing. Then with the exclusion of fetal chromosomal abnormalities, maternal plasma samples of pregnant women carrying suspected skeletal dysplasia, as well as healthy controls (n = 15), were tested by MALDI-TOF MS. The pathogenic variants in suspected fetuses would be confirmed by sanger sequencing and test performance was evaluated.

Results: All of fetal karyotypes were normal. MALDI-TOF MS showed in these families with unaffected parents, eight may respectively have a de novo *FGFR3* c.1138 G>A mutation and two no c.1138 G>A mutation. Sanger sequencing of a family tree were carried out to identify the pathogenic variants. Our method identified all fetal *FGFR3* mutant alleles from maternal plasma, with no false positive results. The sensitivity and specificity of our method were 100% and 100%, respectively.

Conclusion: These preliminary results demonstrate the feasibility of noninvasive prenatal diagnosis of fetal achondroplasia from maternal plasma in Chinese population using MALDI-TOF Mass Spectrometry.



B-155

Algorithm of Suboptimal Health Status, Serum Magnesium and Calcium Levels as a Novel Approach for Prediction and Identification of Pregnant Women Likely to Develop Preeclampsia and Adverse Perinatal Complications in a Ghanaian Population

E. O. Anto[1], P. Roberts[1], C. A. Turpin[2], D. Coall[1], Y. Wang[3], W. Wang[1]. *[1]Edith Cowan University, School of Medical and Health Sciences, Perth, Australia, [2]Komfo Anokye Teaching Hospital, Department of Obstetric and Gynaecology, Kumasi, Ghana, [3]3Beijing Key Laboratory of Clinical Epidemiology, School of Public Health, Capital Medical University, Beijing, China, Beijing, China*

Background: Preeclampsia (PE) is among the leading cause of maternal and foetal morbidity and mortality associated with adverse perinatal complications. The most devastating are pregnant women who are yet to be diagnosed but complain of poor health-suboptimal health status (SHS). For the first time, we explored the usefulness of a SHS questionnaire-25 (SHSQ-25) along with serum magnesium and calcium levels for prediction and early identification of normotensive pregnant women who later develop PE and adverse perinatal outcome. **Methods:** This longitudinal nested case-control study was conducted at the Komfo Anokye Teaching Hospital, Kumasi Ghana. 593 normotensive pregnant women (NTN-PW) with no history of clinical diagnosed disease in the last 3 months were recruited at 10-20 weeks (median 17 weeks) gestation. A validated SHSQ-25 made up of five-domains (namely, fatigue, cardiovascular system, digestive system, immune system and mental health) was used to assess their overall health status. NTN-PW were categories into high SHS and low SHS based on whether their overall SHS score was > and ≤ the median score of the overall SHS score respectively. NTN-PW were then followed until birth (32-42 weeks) and finally, 498 participants [248 high SHS and 250 low SHS] completed the study. Out of the 498 participants, 197 developed PE, 301 were normotensive and 95 were lost to follow-up. Venous blood and urine samples were collected from each participant. Sociodemographic data, obstetric-related characteristics, blood pressure, BMI, albuminuria, serum levels of magnesium and calcium were assessed. **Results:** The mean age of pregnant women was 29.64 years. Out of the 498 NTN-PW who completed the study, 49.8% [248/498] had high SHS of which higher proportion [61.7% (153/498)] later developed PE and lower proportion [38.3% (95/498)] were NTN-

PW. 50.2% [250/498] had low SHS of which lower proportion [(17.6%)44/498] later developed PE and higher proportion [82.4% (206/498) were NTN-PW. Compared to low SHS NTN-PW, high SHS pregnant women who later developed PE (PWLD-PE) had significantly higher systolic blood pressure (SBP) (118.3 vs. 112.4; p<0.0001), diastolic blood pressure (DBP) (75.05 vs. 69.91; p=0.0002), but lower magnesium (Mg) (0.88 vs. 0.96; p=0.0082) and albumin-adjusted calcium (Alb-adj Ca) (2.03 vs. 2.20; p=0.0004) levels at 10-20weeks gestation. Among PWLD-PE, high-normal BP [adjusted odds ratio (aOR), 6.84(1.94-15.40), p=0.0314], low Mg [aOR=5.22(1.09-12.20), p=0.0308) and low Alb-adj. Ca [aOR=2.70(1.29-5.63), p=0.0095] significant independent risk factors for high SHS. At 10-20 weeks gestation, an algorithm of high SHS score with low Mg and low Ca levels level yielded a significant aOR, sensitivity, sensitivity and likelihood ratio (LR) for prediction of PE [aOR=7.51 (2.98-9.41); 77.7%; 88.4% and 2.26], PE + intrauterine growth restriction (IUGR) + low birth weight (LBW) [aOR=5.23(3.08-8.87); 71.4%; 77.7; 2.21], PE + stillbirth + APGAR score <7 after 5min [aOR= 5.62(2.60-12.12); 72.2; 78.4%; 2.28], PE + haemolysis elevated liver enzymes and low platelet count (HELLP) syndrome [6.47(2.49-16.83); 75.0%; 78.3%; 2.27]. **Conclusion:** The SHSQ-25 can be incorporated and utilised as an ideal approach for predictive, preventive and personalised medicine (PPPM) in pregnancy to identify early risk of poor health and likelihood of pregnancy complications.

B-156

Association between High Suboptimal Health Status and Imbalance in Clinicobiochemical Profile, Oxidative Stress Biomarkers and Angiogenic Growth Mediators in Early Normotensive Pregnancy as a Potential Assessment of Cardiometabo-vascular Risk and Endothelial Dysfunction: A Cross-sectional Study in a Ghanaian Population

E. O. Anto[1], P. Roberts[1], C. A. Turpin[2], D. Coall[1], E. Adua[1], Y. Wang[3], W. Wang[1]. *[1]School of Medical and Health Sciences (SMH), Edith Cowan University (ECU), Perth 6027, Western Australia, Perth, Australia, [2]Komfo Anokye Teaching Hospital, Department of Obstetric and Gynaecology, Kumasi, Ghana, [3]Beijing Key Laboratory of Clinical Epidemiology, School of Public Health, Capital Medical University, Beijing, China, Beijing, China*

Background: Suboptimal health status (SHS), which is a state between health and diseases has been associated with cardiovascular risk and endothelial dysfunction. Increased reactive oxygen species (ROS) production and incomplete placental angiogenesis are independent risk factors for endothelial dysfunction (ED) signifying that SHS may play important role in this development. However, these factors have not been studied in parallel. We evaluated a clustered association of SHS-questionnaire-25 (SHSQ-25) along with clinicobiochemical, oxidative stress (OS) biomarkers and angiogenic growth mediators (AGMs) measure as potential risk assessment of CMV and ED among normotensive pregnant women (NTN-PW).

Methods: In this hospital-based cross-sectional study a total of 593 normotensive pregnant women with no history of clinical diagnosed disease in the last 3 months and were visiting the antenatal clinic at the Komfo Anokye Teaching Hospital (KATH) were recruited. SHS was measured using a SHSQ-25. NTN-PW were categorised into high SHS (Poor health) and low SHS (Optimal health) based on whether they their overall SHS score is > and ≤ to the median of the overall SHS sore respectively. Fasting venous blood and urine samples were collected from each participant. Sociodemographic data, blood pressure, albuminuria, obstetric-related characteristics, body mass index (BMI) were assessed. Routine biochemical profile using automatic clinical chemistry analyser, and OS biomarkers [8-epipostaglandin F2 alpha, 8-hydroxydeoxyguanosine and total antioxidant capacity] and AGMs [placenta growth factor (PlGF), vascular endothelial growth factor (VEGF-A), soluble vascular endothelial growth factor receptor-1 (sVEGFR-1/sFlt-1), soluble endoglin (sEng)] were measured using ELISA based technique

Results: The mean age of pregnant women was 29.68 years. The adjusted odds ratios (aOR), 95% confidence interval and p-values indicated that high normal BP [aOR=5.96, 95% CI (2.39-14.85); p<0.0001], low Magnesium [aOR=4.47, 95% CI (3.16-10.15); p<0.0001], low Calcium [aOR=2.19, 95% CI(1.19-5.03), p<0.0001], high LDH [aOR= 2.75(1.60-5.07), p=0.0006], high AST [aOR=2.22(1.68-8.14), p=0.0018], high creatinine [aOR=3.15, 95% CI (1.55-7.04), p=0.0028], Anaemia [aOR=1.58, 95% CI (1.11-2.62), p=0.0397] , high TG [aOR=2.14, 95% CI (1.08-4.79), p=0.0206] and low HDL-c [aOR=2.57, 95% CI (1.15-7.05), p=0.0418] were significant independent risk factors associated with High SHS. There was a significantly negative association between high SHS and serum PlGF (β= -0.188; R^2=3.5%; p=0.0011) and serum VEGF-A (β= -0.143; R^2=2.1%; p=0.0134) but a significantly positive with serum sEng (β= 0.130; R^2=1.7%; p=0.0247), serum sFlt-1 (β= 0.240; R^2=5.7%; p<0.0001), serum 8-epiPGF2α (β= 0.179; R^2=3.0%; p=0.0030), urine 8-OHdG (β= 0.199; R^2=4.0%; p=0.0006), serum 8-OHdG (β= 0.214; R^2=4.6%; p=0.0002). The 1st quartiles and 2nd for serum levels of PIGF [aOR=2.79; 95% CI (1.43 to 3.28); p=0.0002] and [aOR=2.48; 95%CI (1.28 to 5.29); p=0.0154)] respectively, 1st quartiles for VEGF-A [aOR=5.35; 95%CI (2.85 to 10.01); P<0.0001], and the 4th quartiles for sEng [aOR=4.31; 95% CI (2.37 to 7.81); p<0.0001], sFlt-1[aOR=1.84; 95% CI (1.15 to 2.83); p=0.0013], 8-epiPGF2α [aOR=2.23; 95% CI (1.41 to 3.46); p=0.0001], urine 8-OHdG [aOR=1.90; 95% CI (1.28 to 2.83); p=0.0018], and serum 8-OHdG [aOR=1.95; 95% CI (1.30 to 2.90); p=0.0004] were significantly associated with increased odds for high SHS.

Conclusion: Integration of SHSQ-25 in early antenatal health screening will be useful to identify and prevent risk of CMV risk and ED.

B-157

First Trimester Downs Syndrome Screening Using Maternal Serum Pregnancy-associated Plasma Protein A, Free-β Human Chorionic Gonadotrophin, Placental Growth Factor, and α-fetoprotein

S. Das[1], M. Dixit[2]. *[1]Ram Manohar Lohia Hospital, New Delhi, India, [2]Quest Diagnostics, Gurgaon, India*

Background: In the past decade there have been one of the major developments in prenatal screening was two new maternal serum markers of Down syndrome, placental growth factor (PlGF) and α-fetoprotein (AFP) which have become available in the first trimester. A first-trimester serum-only screening test based on existing markers, PAPP-A, free β-hCG, together with PlGF and AFP, might have a discriminatory power equivalent to a second-trimester Quad test. The aim of this study was to assess the screening performance for Down syndrome using first trimester screening (FTS) and two additional markers, serum placental growth factor (PlGF) and α-fetoprotein (AFP). **Methods:** This is a pilot retrospective case-control study of 1013 unaffected pregnancies and 67 pregnancies affected by Down syndrome. Serum samples were tested for pregnancy-associated plasma protein A (PAPPA), free-β human chorionic gonadotrophin, placental growth factor(PLGF), and α-fetoprotein(AFP), and results were expressed as multiples of median (MoM). Multivariate Gaussian modeling was used to calculate risks for different combinations of markers and to predict the detection rate (DR) and false positive rate (FPR). The predicted performance of enhanced FTS (FTS plus PlGF and AFP) was compared with usual FTS; the performance with and without nuchal translucency (first trimester quad) was also assessed. **Results:** For the pregnancies affected with downs syndrome, the median PlGF level was 0.622 MoMand median AFP 0.764 MoM. Adding PlGF and AFP improved the screening performance. At 5% FPR, DR increased by 3.8 % from 89.6% to 93.4% using enhanced FTS combined with nuchal translucency. When assessed without the nuchal translucency at 5 % FPR the DR using enhanced FTS was 81.8% **Conclusion:** The performance of FTS is enhanced by adding PlGF and AFP both with and without nuchal translucency.

B-158

Telemedicine Screening Adolescent Metabolic Syndrome in Greek Schools

F. Bacopoulou[1], V. Efthymiou[1], G. Palaiologos[2], I. Papassotiriou[2]. *[1]Center for Adolescent Medicine and UNESCO Chair on Adolescent Health Care, First Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece, [2]Department of Clinical Biochemistry, "Aghia Sophia" Children's Hospital, Athens, Greece*

Background: The worldwide prevalence of Metabolic Syndrome (MetS) in children and adolescents varies greatly from 0-50%, depending on the ethnicity, the diagnostic criteria and the presence and degree of obesity. The most clinically used definitions for adult MetS are those set by the International- Diabetes-Federation (IDF) and the National-Cholesterol-Education-Program-Third-Adult-Treatment-Panel (NCEP:ATPIII). There is still no single precise definition of the MetS in adolescence and the contribution of the adult components of MetS, to facilitate early diagnosis in adolescents, is of much debate. Accepted definitions of MetS in youth include the most recent consensus by the IDF for MetS in children and adolescents as well as the NCEP:ATPIII definition with modified cut-off values for youths. Telemedicine services have been effective in addressing and improving youth health status in schools. The aim of this study was to screen for the metabolic syndrome a regionally representative adolescent sample, for the first time in Greek schools with the use of telemedicine. The associations of MetS with anthropometric, blood chemistry, sociodemographic and behavioral parameters were also explored.

Methods: A total of 2,100 high school adolescents were initially contacted and finally 1,578 adolescents participated in the program (75.1% response rate). Participants'

mean age±SD was 14.4±1.7years (42.5% boys, 57.5% girls). The Vida24 mobile health tele-monitoring service was used which enabled remote monitoring of participants' data recorded via tele-monitoring devices (weight scale, blood pressure device, blood glucose and triglycerides device) through 3/4G and Wi-Fi networks. Other blood chemistry parameters such as total-Cholesterol and HDL-Cholesterol measurements were performed in the Clinical Biochemistry Lab.

Results: The prevalence of MetS in 1,578 adolescents (14.4±1.7 years) was 2.6% (3.4% among males; 2.0% among females), highest (4.3%) at age 13 years and lowest (1.3%) at 16 years. Adolescents with MetS had significantly higher mean body mass index (BMI)±SD than those without MetS (30.2±4.2 vs.21.3±3.2Kg/m^2, respectively, p<0.001); among participants with obesity, 31.6% had MetS. Abdominal obesity, elevated triglycerides, low HDL-cholesterol, impaired fasting blood glucose (FBG) and elevated blood pressure (BP) were detected in 9.5%, 2.3%, 10.7%, 25.9% and 21.8% of participants, respectively. Additional analysis (modified NCEP:ATPIII youth criteria) demonstrated similar overall prevalence of MetS (2.9%). Statistically significant correlations were found between most anthropometric and MetS characteristics, with the exception of FBG, which was correlated only with systolic BP. BMI was strongly correlated with waist and hip circumferences (r=0.818, p<0.001 and r=0.825, p<0.001, respectively). Single parenthood and older maternal age (>60 years) were risk factors for MetS. Although counterintuitive, body image distortion, body dissatisfaction and bullying about weight were more prevalent in normal weight girls.

Conclusion: In this study, screening for the metabolic syndrome through telemedicine in the adolescent-friendly school setting, was effective, as the majority of adolescents and their parents agreed to participate. On the basis of two definitions, the IDF and NCEP:ATPIII for youth, 2.6% and 2.9% of high school students, respectively, were identified as having MetS. Although the overall prevalence of MetS was low, it was increased by 12-fold when obesity was taken into account.

B-159

Evaluating Hemoglobin A_{1C} Devices for Testing at the Point of Care in a Hub and Spoke Laboratory Model

J. L. Robinson, J. L. Gifford. *Alberta Public Laboratories, Calgary, AB, Canada*

Background: The percentage of glycated hemoglobin A (HbA_{1c}) is a robust marker of a patient's mean blood glucose concentration over the previous 90 days used to inform diabetes management. For most non-pediatric patients, there is no strict turnaround time requirement for HbA_{1c} reporting; and patient testing from a large geographic area can be fulfilled by high volume instruments in a single hub laboratory. However in pediatric patients, the frequent HbA_{1c} testing required for diabetes monitoring can be burdensome due to the challenges of phlebotomy in children and inconvenience to the caregiver. As a consequence, pediatric patients often present to their endocrinologist without the necessary test results. Therefore point of care (POC) HbA_{1c} testing is an attractive option for managing this population, despite access to a high volume instrument. **Objective:** To evaluate two Health Canada approved POC HbA_{1c} devices, the Roche Diagnostics cobas b101 and the Siemens Medial Solutions Diagnostics DCA Vantage, for implementation into a pediatric endocrinology clinic. The devices were assessed for ease of use, analytical performance, and result correlation to a high volume analyzer (Roche Diagnostics c513) located in the hub laboratory. **Methods:** The evaluation was performed according to CLSI guidelines. Within-run and between-run precision were assessed with manufacturer and third-party QC material (BioRad Laboratories). Device accuracy was determined using stored National Glycated Standards program (NGSP) reference material from the College of American Pathologists. Device linearity was determined through dilution of high and low patient specimens and assessed using linear regression. Patient comparisons (N=20) using discarded EDTA samples were performed between the b101 or DCA Vantage and the Roche Tina-quant Hemoglobin A1cDx Gen.3 assay on the c513. In the statistical analysis, $p<0.05$ was considered significant. **Results:** Both the cobas b101 and the DCA Vantage measure %HbA_{1c} in a drop of blood using a cartridge based, latex agglutination inhibition immunoassay. The precision of the b101 at normal and pathological levels of %HbA_{1c} was 1.8-2.4 %CV for within-run and 3.2-4.3 %CV for between-run. For the DCA Vantage, precisions of 1.4-7.3 %CV for within-run and 1.9-3.8 %CV for between-run were observed. In accuracy experiments, average absolute biases of -1.0 and +0.1 %HbA_{1c} were observed for the b101 and DCA vantage, respectively. The b101 was linear from 4.2-12.4 %HbA_{1c} and the DCA Vantage from 3.9-13.3 %HbA_{1c} ($p<0.05$). Patient comparisons demonstrated a linear relationship between the b101 or DCA Vantage and the c 513 assay ($p<0.05$), however, both instruments demonstrated a negative bias (mean absolute biases of -0.6 and -0.2 %HbA_{1c} for the b101 and DCA Vantage, respectively). When compared to the c513, both instruments showed larger discrepancies at greater %HbA_{1c} values, and the negative bias was especially prominent for the b101. Because a change of 0.5 %HbA_{1C} can be interpreted as clinically significant, these analytical discrepancies may impact patient management. **Conclusion:** Although, POC HbA_{1C} devices offer increased efficiencies to patients and their caregivers, physicians must be aware that analytical differences may exist between these devices and high volume analyzers in a hub and spoke laboratory model.

B-160

Comparison of Roche Cobas e601 and Brahms Kryptor in Combined First Trimester Screening for Fetal Aneuploidy

M. H. Lim, R. Tan, C. H. C. Tan, C. P. Yeo. *Singapore General Hospital, Singapore, Singapore*

Background: With a relatively good detection rate between 82-87%, combined first trimester screening remains one of the most widely used screening test for trisomy 21 (T21), trisomy 18 (T18) and trisomy 13 (T13) in developed countries. Measurements of the biochemical serum markers, free-beta human chorionic gonadotropin (f-βHCG) and pregnancy associated plasma protein A (PAPP-A), are used in conjunction with nuchal translucency findings on the ultrasound to calculate a risk profile of the fetus for T21, T18 and T13 that is adjusted for maternal age. This study aims to evaluate and compare the performance of the Roche Cobas e601 platform against our current Brahms Kryptor for measurement of f-βHCG and PAPP-A, calculation of the respective multiples of median (MoM), and risk classification of T21, T18 and T13.

Methods: Imprecision, linearity verification, limit of detection and carryover for the Cobas e601 platform were performed in accordance to the laboratory's method evaluation protocol that is adapted from the Clinical & Laboratory Standards Institute (CLSI) guidelines. A total of 444 singleton pregnant women were recruited from the hospital's first trimester clinic between the period May 2017 to June 2018. Serum f-βHCG and PAPP-A, routinely assayed on the Kryptor analyser, were additionally analysed on the Cobas e601 system for this study. Nuchal translucency measurements were obtained by sonographers from the Department of Obstetrics and Gynaecology as per usual practice. A final number of 433 samples were analysed in our study. Risk scores were calculated using the Astraia software provided by the Fetal Medicine Foundation.

Results: Manufacturer's claim for linearity, limit of detection and carryover were verified for both f-βHCG and PAPP-A on the Cobas e601. Within-run and total imprecisions for both analytes were within the claims, with coefficient of variation (CV) between 1.0-2.1%. MoM for f-βHCG on Cobas, was on average, 0.15 MoM (95% CI = 0.11 to 0.19) higher than on Kryptor (p<0.0001). In contrast, MoM for PAPP-A on Cobas was on average, 0.18 MoM (95% CI = 0.16 to 0.20) lower than on Kryptor (p<0.0001). At a cut-off of 1:300, good agreement for the risk classification of T21, T18 and T13 were observed between the two platforms, with levels achieving at least 98.6% on the 2x2 agreement. 6 and 2 cases reported as high risk on the Cobas platform for T21 and T13 respectively were however classified as low risk by results from the Kryptor platform.

Conclusion: Despite significant differences between the MoM for both f-βHCG and PAPP-A on the two platforms, we established that the resultant risk classifications for T21, T18 and T13 would be the same for majority of cases. Our observation of an increased trisomies pick up rate by the Cobas, as compared to Kryptor, will require additional pregnancy outcome data to determine if that translates to a better detection rate or a higher false positive rate for the Cobas. Nevertheless, a switch to the Cobas e601 system from our current Brahms Kryptor can be made with minimal impact to the clinical interpretation of T21, T18 and T13 risk scores.

B-162

Performance Evaluation of the Atellica CH TBil_2 Assay to Include Neonates*

J. T. Snyder, J. Koellhoffer. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to add neonatal claims to the Atellica® CH TBil_2 Assay and to evaluate the analytical performance of these claims on the Atellica® CH Analyzer. Total Bilirubin (TBil_2) is formed by the breakdown of hemoglobin by the liver. In neonates, particularly those born prematurely, increased bilirubin is often due to the immature liver being unable to produce the enzyme glucuronosyltransferase, which assists in the breakdown of bilirubin. If bilirubin accumulates to toxic levels, the newborn is at risk for brain damage and other complications. The Atellica CH TBil_2 Assay uses vanadate to oxidize both conjugated (direct) and unconjugated bilirubin. This reaction causes a reduction in the optical density of the yellow color specific to bilirubin. The decrease in optical density is proportional to the concentration of total bilirubin in the sample.

Methods: Performance testing included method comparison, detection capability, interference testing, and reference range. Method comparison studies were conducted according to CLSI EP09-A3, with neonatal patient sample results compared with results from the ADVIA® Chemistry 1800 TBIL_2 assay. Limit of quantitation (LoQ) testing was tested in accordance with CLSI EP17-A2, where the lowest sample concentration meeting the maximum allowable imprecision and maximum allowable bias acceptance criteria was taken as the LoQ estimate. Interference testing was tested according to the governing standard CLSI EP07-A2. Reference range was conducted according to EP28-A3c for multiple age groups and evaluated against an existing reference range found in current literature.

Results: The method comparison study yielded a regression equation of y = 1.00x + 0.1 mg/dL, with r of 0.983. The observed LoQ was 0.1 mg/dL, with a total error of 0.0 mg/dL. Interference testing was conducted for fetal hemoglobin (HbF), indican, and cyanokit. TBil_2 was tested at two different analyte concentrations for each interferent. For HbF, interference ranged from -6 to -9%, with higher interference occurring at a lower concentration of TBil_2. Interference for indican was 0% and ranged from 0 to -2% for cyanokit. TBil_2 reference ranges were verified from the current literature and are as follows: 0-1 day old: <8.0 mg/dL; 1-2 days old: <12.0 mg/dL; 3-5 days old: <16.0 mg/dL; 5 days-60 years old: 0.3-1.2 mg/dL; 60-90 years old: 0.2-1.1 mg/dL; >90 years old: 0.2-0.9 mg/dL. Less than 10% of samples from each age group were outside the claimed reference range.

Conclusion: The Atellica CH TBil_2 Assay tested on the Atellica CH Analyzer demonstrated acceptable detection capability and interference testing. An existing reference range for neonates and adults was verified with acceptable results. Method comparison results showed acceptable agreement compared to the ADVIA® Chemistry 1800 TBIL_2 assay. *Product availability varying by country per regulatory requirements

B-163

Determination of Vitamin K_1 and Vitamin K_2 (MK-4, MK-7) in Serum of Patients with Cystic Fibrosis by New LC-MS/MS Method

R. Prusa, J. Cepova, E. Klapkova, K. Dunovska. *University Hospital Motol, Prague 5, Czech Republic*

Background: Vitamins K_1 and K_2 are the cofactors for the enzyme gamma-glutamylcarboxylase, which is involved in carboxylation of the vitamin K-dependent proteins. Vitamin K deficiency is frequently observed in patients with cystic fibrosis due to bowel resection, malabsorption of fat, liver disease, etc. Objective of this study was to determine the levels of vitamin K in the paediatric patiens with cystic fibrosis by a new LC-MS/MS method.

Methods: A UPLC-tandem mass spectrometry method for the determination of vitamin K_1 and vitamin K_2 (MK-4 and MK-7) in human serum was developed. Mass spectrometric detection was performed on a triple-quadrupole in the positive elecrospray ionization mode by multiple reaction monitoring (Agilent 1290 with Triple Quad 6470, Agilent Technologies), d7-MK-7, d7-MK-4, and d7-K_1 was used as internal standards. A solid phase extraction was required for the sample clean up. Chromatographic separation was carried out on SB-C8 column (2.1x100 mm, 1.8 µm, Agilent Technologies) using a binary gradient of mobile phases (A - 0.1% ammonium fluoride in 50% methanol, B - 0.1% ammonium fluoride in methanol). The separation was accomplished at 40°C. The analysis time was 9 minutes. We measured samples from 20 paediatric patients with cystic fibrosis and compared the results of healthy population.

Results: The LC-MS/MS method has been successfully validated. Lower limits of quantification were 0.03 ng/mL for K_1 and MK-4, and 0.05 ng/mL for MK-7. The intra and inter-day accuracy and precision were evaluated on two QC samples by multiple analysis and coefficients of variation were 2.3-7.0% for intra-assay, 7.2-11.3% for inter-assay, and 0.5-6.6% for precision. No interference was found between K_1, menaquinone-4 and menaquinone -7, or deuterated internal standards. Serum levels of vitamin K_1, menaquinone-4 and menaquinone-7 in patients with cystic fibrosis were (median ± SEM): **0.054** ± 0189 ng/mL; **0.003**±0.033 ng/mL; and **0.124**±0,018 ng/mL. Serum levels of vitamin K_1, menaquinone-4 and menaquinone-7 in healthy population were (median ± SEM): **0.412**±0.150 ng/mL; **0.063**± 0.021 ng/mL; and **0.309**±0,146 ng/mL.

Conclusion: We developed and fully validated a new LC-MS/MS method for determination of vitamin K1, MK-4 and MK-7. The comparison of vitamin K levels in patients with cystic fibrosis and healthy population showed statistically significant differences. Vitamin K supplementation in patients with cystic fibrosis seems to be necessary and essential.*Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic)*

B-164

A Simulation Study to Assess the Effect of Analytic Error on Neonatal Glucose Measurements using Canadian Pediatric Society Position Statement Action Thresholds

M. E. Lyon, K. Parker, L. Strueby, M. Inman. *Saskatchewan Health Authority, Saskatoon, SK, Canada*

Background & Objective: The Canadian Pediatric Society (CPS) has recently endorsed an algorithm for the screening and immediate management of babies at risk of neonatal hypoglycemia that provides time dependent glucose concentration action thresholds. The objective of this study was to evaluate the impact of glucose analytic error (bias and imprecision) on the misclassification of glucose meter results from a neonatal intensive care unit (NICU) using the CPS guidelines.

Methods: One year of NICU glucose meter results (n=23,749) from Royal University Hospital in Saskatoon, Saskatchewan were analyzed. No patient identifiers were extracted. These data were treated as "true" results, devoid of error, and were categorized according to CPS hypoglycemia decision-making threshold values. Equation [1] describes the introduction of error into the dataset and the newly perturbed values were re-categorized according to CPS thresholds and then compared to the original category of the "true" value.

[1] *Error Gluc = Original Gluc + (Original Gluc * CV * U[0,1]) + (Original Gluc * BIAS)*

The percentage of the simulated values that were misclassified at CPS hypoglycemia thresholds - 1.8 mmol/L (32.4 mg/dL), 2.0 mmol/L (36 mg/dL) and 2.6 mmol/L (46.8 mg/dL) - were determined and represented as contour maps.

Results:

Conditions	Action	Threshold (mmol/L)					
		< 1.8	≥ 1.8	< 2.0	≥ 2.0	< 2.6	≥ 2.6
Original Data Set	N/A	215 (0.9%)	23,264 (99.1%)	357 (1.5%)	23,122 (98.5%)	1292 (5.5%)	22,187 (94.5%)
Simulated Scenario #1 5% CV + 0.25 mmol/L Bias	Failure to treat	145 (0.6%)	23,334 (99.4%)	240 (1.0%)	23,239 (99.0%)	658 (2.8%)	22,821 (97.1%)
Simulated Scenario #2 5% CV - 0.25 mmol/L Bias	Over-treat	403 (1.7%)	23,076 (98.3%)	686 (2.9%)	22,793 (97.1%)	2,137 (9.1%)	21,342 (90.9%)

Table 1: Simulated number of glucose results misclassified according to CPS action thresholds as a function of glucose analytic error.

Conclusions: Using CPS action thresholds, the simulation model predicted a significant proportion of neonates are at risk of inappropriate clinical action - both of omission or "failure to treat" and commission or "over-treatment" - in response to routine NICU blood glucose meter results.

B-165

Pediatric Reference Intervals for 17 Roche Cobas 8000 e602 Immunoassays in the CALIPER Cohort of Healthy Children and Adolescents

M. Bohn[1], V. Higgins[1], S. Asgari[1], B. Hoffman[2], K. Adeli[1]. *[1]The Hospital for Sick Children, Toronto, ON, Canada, [2]Mount Sinai Hospital, Toronto, ON, Canada*

Background: The diagnostic utility of laboratory testing in pediatric medicine relies heavily on the availability of appropriate reference intervals. The Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) has successfully established a comprehensive database of covariate-stratified reference intervals for many pediatric laboratory tests using a large, healthy reference population. While several automated analyzers in widespread use in clinical laboratories have already been studied by CALIPER, lack of immunoassay standardization and marked intermethod differences among manufacturers necessitates direct reference interval establishment for each major analytical platform. Here, we report, for the first time, comprehensive pediatric reference intervals for 17 endocrine and special chemistry markers on the Roche cobas 8000 e602 analyzer.

Methods: Healthy children and adolescents (0 to <19 years) were recruited through community initiatives in the Greater Toronto Area and Hamilton regions as part of the CALIPER study. Inclusion was assessed through a detailed health questionnaire

and participants with acute or chronic illnesses and/or recent medication use were excluded from analysis. Serum samples from a total of 601 healthy participants were analyzed for 17 endocrine and special chemistry markers on the Roche cobas 8000 e602 analyzer. Analyte concentrations were visually assessed and age- and sex-specific partitions were confirmed using the Harris & Boyd statistical method. Outliers were subsequently identified and removed using the Tukey or adjusted Tukey method for Gaussian and non-Gaussian data distributions, respectively. In accordance with Clinical and Laboratory Standards Institute EP28-A3c guidelines, reference intervals were established using the nonparametric method (n≥120) or the robust method of Horn and Pesce (40≤n<120), and corresponding 90% confidence intervals were calculated.

Results: Reference values for all analytes measured required age partitioning, particularly during critical periods of growth and development such as early life and adolescence. Of the 17 analytes measured, 8 required sex partitioning, including estradiol, ferritin, follicle stimulating hormone, luteinizing hormone, progesterone, testosterone, thyroid stimulating hormone, total triiodothyronine and sex-hormone binding globulin.

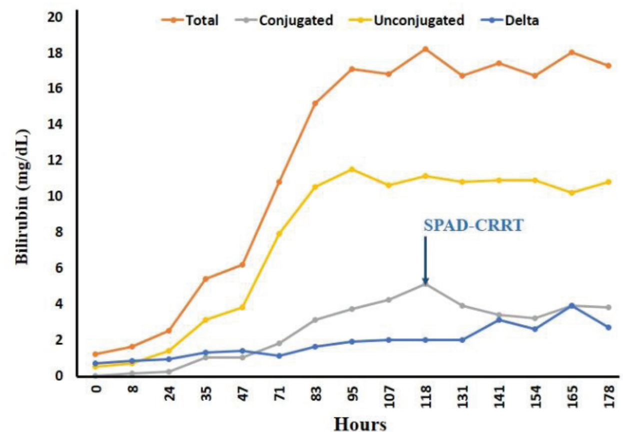
Conclusion: This is the first comprehensive study to determine robust pediatric reference intervals for Roche immunoassays. Reference intervals were generally similar to those previously published by CALIPER on other analytical platforms (i.e. Abbott Laboratories, Beckman Coulter Diagnostics and Ortho Clinical Diagnostics), highlighting the reproducibility of age- and sex-specific trends in reference values observed across the pediatric age range. The reference intervals established in this study will contribute to improved accuracy of test result interpretation and clinical decision making in clinical laboratories utilizing Roche immunoassays globally.

B-166

Why My Patients Conjugated Bilirubin is Unexpectedly High Despite Utilizing Single-Pass Albumin Dialysis with Continuous Renal Replacement Therapy (SPAD-CRRT)?

U. Garg, A. Wiebold, A. Ferguson, V. Chadha. *Children's Mercy Hospital, Kansas City, MO*

Background: We have previously shown that single-pass albumin dialysis with CRRT (SPAD-CRRT) can be successfully utilized in the management of patients with acute liver failure (ALF) and acute kidney injury (AKI). With SPAD-CRRT bilirubin clearance (a surrogate marker for albumin-bound toxins) was >10-fold as compared to CRRT only. However, we failed to observe increased bilirubin clearance in a group of ALF patients who received SPAD-CRRT. **Objective:** To investigate failure of bilirubin clearance in certain ALF patients. **Material and Methods:** We studied three patients with ALF who failed to show expected high bilirubin clearance. Bilirubin fractions were measured or calculated using Vitros 5600 chemistry analyzer. This method is unique and uses two slides to measure total, unconjugated and conjugated-bilirubins, and calculates delta bilirubin. Delta-bilirubin is a conjugated-bilirubin which is covalently bound to albumin. Since most instruments do not measure or calculate delta-bilirubin, in general Vitros users do not report delta-bilirubin values as a separate fraction, but as a part of conjugated-bilirubin. This provides conjugated and unconjugated-bilirubin values comparable to other chemistry analyzers. **Results and Discussion:** All three patients had significant unconjugated hyperbilirubinemia (37 to 70% of total bilirubin). SPAD-CRRT didn't clear unconjugated-bilirubin as it is tightly bound to albumin. In contrast to most patients with ALF, we also found significantly decreased clearance of conjugated-bilirubin in these patients. Further investigation by reviewing the raw data showed that this fraction of bilirubin was delta-bilirubin (Figure), and was not being cleared by SPAD-CRRT as it is albumin-bound. This fraction accumulates in the patients with prolonged liver failure and biliary atresia. **Conclusions:** Decreased clearance of conjugated-bilirubin in ALF patients was due to increased delta-bilirubin. Since most laboratories do not measure or report delta-bilirubin, increased delta-bilirubin may lead to the perception that SPAD-CRRT is not working, and may lead to unnecessary and expensive work-up.



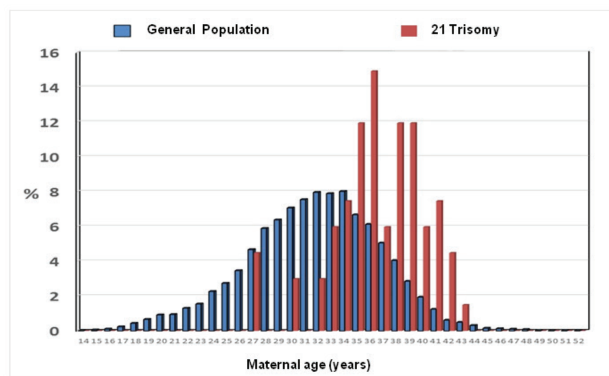
B-167

Retrospective Analysis of an Observational Study in 20000 Pregnancies Undergoing First-trimester Screening with Biochemical Markers: Pregnancy Associated Plasma Protein A (PAPPA) and Beta Free the Chorionic Gonadotropin (FBHCG) and Sonographic Parameters

A. Kozak, M. Viale, H. Aiello, M. Serra, E. Miler, G. Rubino, P. Fainstein Day. *Hospital Italiano de Bs. As., CABA, Argentina*

Aneuploidies are the most common genetic abnormalities detected during prenatal care and include chromosomes 21 (Down's syndrome, T21), 18 (Edward's syndrome, T18) and 13 (Patau´s syndrome, T13). The combined first-trimester screening incorporates maternal age, fetal nuchal translucency (NT) and biochemical markers: PAPPA and FBHCG. Since the introduction of this test, have shown the association between low levels of PAPP-A and high levels of FBHCG with high gestational risk. Together, estimate the fetal risk to develop the disorders above. The Multiples of the Median (MoM) values are calculated using a database with adjusted data for gestational age, multiple pregnancy and systematic differences between laboratories and assay reagents. Objective: to describe the association between the frequency of aneupolidies and maternal age between 11 and 14 weeks of pregnancy in samples collected in Buenos Aires, Argentine between Feb-13 to Feb-18. Methods: FBHCG and PAPP-A levels were performed by chemioluminiscent technology (IMM 2000,Siemens). The ALPHA program (Logical Medical Systems Limited, UK), considers the serum markers levels besides the value of NT. The values are converted by the program to MoM in relation to the fetal length (LCN), and adjusted. Statistic Analysis: Kruskar-Wallis Results: 19,553 patients data were analyzed and median of maternal age (y)/ % aneuploidies were 33 y /0; ≥ 35 y/38% and ≥ 40 y/8%. 99.4% of cases were negatives and 0.6% were positive for the aneuploidies: n: 67 high risk for T21; n: 40 for T18; n: 11for T13. Conclusions: There is a strong association between biochemical markers and nuchal translucency with the most frequent aneuploidies. A higher frequency is also observed with the increase in maternal age.We emphasize the importance of calculating the MoM value for the methodology used in the measurement of biochemical markers.

We highlight the relevance of the sampling used in this study and the importance of prenatal screening



B-168

Stability Study of Maternal Serum Screening Analytes in Serum and Alpha Fetoprotein (AFP) in Amniotic and Cerebral Spinal Fluid

S. L. La'ulu[1], L. Pace[2], J. R. Genzen[3]. [1]*ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT,* [2]*ARUP Laboratories, Salt Lake City, UT,* [3]*Department of Pathology, University of Utah, Salt Lake City, UT*

Background: Sample stability is important to evaluate to ensure accurate results. Manufacturers do not always provide a comprehensive range of stabilities to address all storage conditions that may be encountered. The objective of this study was to evaluate sample stabilities for five maternal screening analytes in serum. Additionally, alpha-fetoprotein (AFP) stability was assessed in amniotic fluid (AF) and cerebral spinal fluid (CSF).

Methods: Stability studies were performed to evaluate ambient, refrigerated, and frozen sample stabilities of AFP, β-human chorionic gonadotropin (βhCG), inhibin A, pregnancy-associated plasma protein A (PAPP-A), and unconjugated estriol (uE3) in serum using the Beckman Coulter UniCel DxI 800 immunoassay system. Additionally, sample stability of AFP in AF and CSF was also evaluated using the DxI.

Residual serum and CSF samples were acquired from storage (-20°C) to create pools (n=3) with analyte concentrations within the assay analytical measurement range. A sufficient number of samples were pooled to enable single-use aliquots for each day of testing. AF samples were acquired from refrigerated storage (2-8°C), using six unique samples (not pooled) with adequate volume and distributed into single-use aliquots. The samples were tested in singlicate for each respective analyte to create a baseline value. Aliquots were stored at ambient (room temperature), refrigerated (2-8°C), and frozen (-20°C) as described below. After storage at the assigned temperatures, samples were tested in singlicate for the respective analytes in the following manner: ambient, once a day for 3 days; refrigerated, once a day for 14 days; and frozen, once a month for 6 months, then at 9 months and 12 months. For AF, samples were tested in singlicate on days 1, 3, 5, 7, 14, 21, and 28, 2 months (refrigerated and frozen), and 3 months (frozen). Percent differences were calculated between the baseline value and the subsequent aliquots. The average % difference was calculated for each pool, followed by the average % difference of all pools combined. Acceptable criteria was no greater than ±15% difference from the baseline result.

Results: The overall average % difference of all pools combined was <10% for all analytes/fluids, thereby meeting the prescribed acceptance criteria. Percent differences ranged from -7.10% (uE3; serum, refrigerated) to 9.17% (AFP; CSF, frozen).

Conclusions: AFP, βhCG, inhibin A, PAPP-A, and uE3 remained stable in serum (and AFP in CSF) for 3 days ambient, 14 days refrigerated, and 1 year frozen. AFP was stable in AF for 28 days ambient, 2 months refrigerated, and 3 months frozen. The timeframe of analyte stability observed in this study may prove useful for situations such as prolonged storage, retesting, and add-on testing, thereby avoiding sample recollection.

B-169

Case Report: 18p- Syndrome due to Translocation (15,18) in a Newborn

A. M. Silva, M. Conchon, J. M. Fenandes. *DASA, Barueri, Brazil*

Background: Jean de Grouchy first described 18p- Syndrome in 1963 with a frequency of 1 to 50.000 live births, and female to male ratio of 3:2, being around 300 cases reported worldwide. We describe a case of deletion of short arm of chromosome 18 due to an unbalanced translocation between chromosomes 15 and 18 in Brazil.

Objective: To describe an unusual and rare karyotype finding with translocation of chromosomes 15 and 18, resulting in monosomy of short arm of chromosome 18.

Methods: A 400-band karyotype was performed in peripheral blood lymphocyte and 20 metaphases were analyzed in an infant male patient with syndromic faces and bilateral cardiopathy.

Results: Twenty metaphases were analyzed from a 1-day old infant peripheral blood lymphocyte and found all cells with a derivative chromosome by the unbalanced translocation between chromosomes 15 and 18, causing monosomy of the short arm of chromosome 18.

Conclusion: The phenotype described in the infant, bilateral cardiopathy and syndromic facies are present on patients with in 18p- Syndrome. Both deletions on short or long arm of 18 chromosome are rare and present facial dysmorphism and mild to severe intellectual deficiency. Since the array examination was not performed, we cannot determine with certainty that the additional material on chromosome 15 is from chromosome 18 and what would be the breaking point. Most of cases reported with 18p- are results from *de novo* mutation, still parents should be investigated to rule out the possibility of heredity translocations.

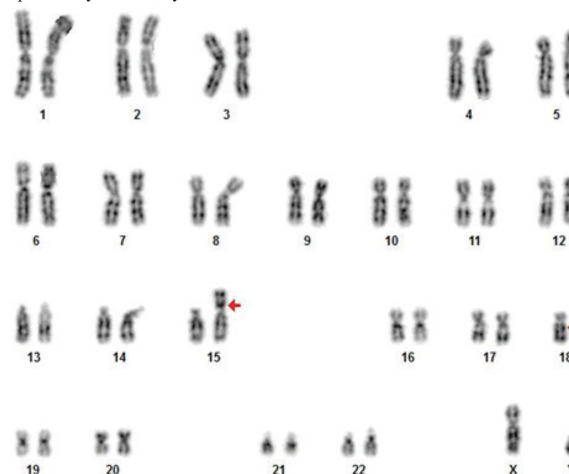


Fig 1. Karyotyping from the peripheral lymphocytes of a 1-day old infant, showing deletion of short arm of chromosome 18 due to a translocation (15;18).

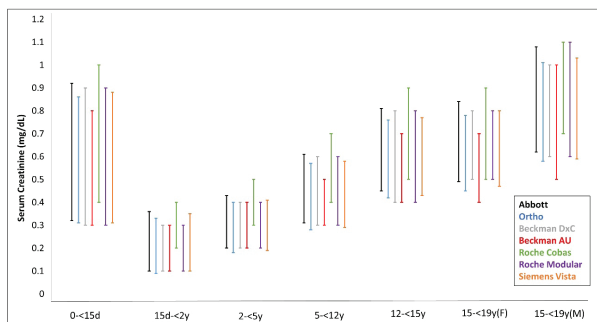
B-170

Assessing the Feasibility of Common Reference Intervals across Different Analytical Platforms: Evidence from CALIPER Pediatric Reference Intervals Database

J. Taher[1], H. Tahmasebi[2], J. Cosme[1], V. Higgins[1], K. Adeli[2]. [1]*University of Toronto, Toronto, ON, Canada,* [2]*The Hospital for Sick Children, Toronto, ON, Canada*

Objectives: Appropriate test result interpretation and clinical decision-making rely on accurately established reference intervals, which have traditionally been lacking for the pediatric population. The Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) project has largely addressed this gap by developing pediatric reference intervals for several major analytical systems. Given minimal analytical differences for many biochemical assays measured on different analytical instruments, we developed and applied a mathematical approach to assess the appropriateness of common pediatric reference intervals. **Methods:** CALIPER reference intervals for 34 chemistry assays across 7 IVD manufacturers were compared. Acceptable bias between instruments was specified as the total allowable error (TEa) minus twice the precision goal as indicated by Institute of Quality Management in Healthcare

(IQMH). Platform-specific reference intervals were compared by assessing the absolute and percent difference between the highest and lowest instrument limit for both upper and lower reference limits. Harmonization was recommended for a given assay if there were minimal differences between most analytical instruments based on acceptable bias across age and sex partitions. **Results:** In an initial analysis of 8 biochemistry assays, five analytes (creatinine, direct bilirubin, total bilirubin, alanine aminotransferase, and magnesium) demonstrated acceptable bias across assays from different manufacturers, thereby resulting in a recommendation for common reference intervals. The Figure depicts one such candidate (creatinine) for common reference intervals based on minimal bias between instruments. Conversely, other analytes (cholesterol, immunoglobulin G, and lactate dehydrogenase) exhibited significant bias across analytical instruments, suggesting the need for platform-specific reference intervals. **Conclusions:** This is the first comprehensive study comparing pediatric reference intervals across analytical systems to propose common reference intervals. Due to minimal bias between platform-specific reference intervals for many chemistry assays, common pediatric reference intervals can be developed, thereby leading to more consistent result interpretation and improved clinical decision making.



B-171

Laboratory Methods in the Diagnosis of Congenital Hypotonia

D. Daniel[1], J. Diks[1], K. Kasbati[2], A. Zuretti[3]. [1]*SUNY Downstate Medical Center, Brooklyn, NY,* [2]*Maimonides Medical Center, Brooklyn, NY,* [3]*SUNY Downstate Medical Center and Maimonides Medical Center, Brooklyn, NY*

Background: Congenital hypotonia is a well-recognized condition that presents in early life. Its etiology can be traced to a number of entities, including genetic, metabolic, neurologic causes. Diagnosis of the cause of hypotonia requires a multifaceted approach. Currently, an algorithm exists for systemic evaluation of a "floppy infant". This workup includes a thorough history and physical examination to form a pathway to diagnosis, with laboratory testing and biopsy information providing guidance. The utility of the tests performed should be taken into consideration, especially the implications of the results on the immediate management of the patient in an inpatient setting as they impact costs. This is a patient born at term, with admission to NICU for evaluation of congenital hypotonia and epilepsy.

Methods: Baby girl, born at 38 weeks and 3 days was admitted to NICU for hypotonia (poor tone, weak cry, cyanotic at birth), low Apgar score (4/6) and intractable seizures. The patient was GJ tube dependent, oxygen dependent and on multiple anticonvulsants. Tests that were ordered for the workup of this patient's hypotonia and epilepsy, in order of send out date, included CSF Amino Acid, CPK isoenzyme electrophoresis, pyruvate, urine amino acid screen, plasma amino acid screen, urine organic acids, plasma acylcarnitine quantitative, Prader-Willi/Angelman methylation analysis, serum fatty acid profile, spinal fluid amino acids, serum pipecolic acid x2, infantile epilepsy panel, urine pipecolic acid, urine alpha aminoadipic semialdehyde, and SNP array comparative genomic hybridization. Subsequently, SLC13A3 and TP53RK gene analysis were ordered.

Results: All send out tests that were ordered, including infantile epilepsy panel, resulted negative. A SNP array showed 20q13.12 duplication of uncertain significance, and urine alpha aminoadipic semialdehyde was still pending at the time of writing, over 1 month after the date of send out. Of note, the SNP array did not result until over 1 month after the date of send out.

Conclusion: The workup of this patient did not adhere with current systematic approach algorithms that exist. These algorithms begin the investigation of hypotonia with the review of the patient and the patient's family history. Subsequently, physical examination markedly narrows the differential. The physical and neurological exam will then dictate the laboratory tests indicated, following the algorithm of suspected metabolic disease, suspected CNS dysfunction, or suspected lower motor neuron disease. In this case, the laboratory investigations that were performed did not follow a single pathway systematically, but rather ordered multiple tests for each of the disease entities. The use of multifaceted and molecular testing did not impact the acute management of the patient. The turnaround time required for SNP array and alpha aminoadipic semialdehyde was too long to be of use Several tests ordered resulted negative and would not have changed the acute inpatient management of the child. The tests ordered did not adhere to protocol consistently, and increased cost. The goal of our report on this rare case is to improve future management and optimize the laboratory diagnosis of cases with similar presentation.

B-172

Reference Range for Ferritin in Healthy Children and Adolescents in the City of Cuiabá, Mato Grosso, Brazil

N. Slhessarenko[1], M. Slhessarenko[2], C. J. F. Fontes[3], G. Campana[4], T. C. Veloso[5], R. S. Azevedo[6], A. Andriolo[7]. [1]*Federal University of Mato Grosso and ALTA-DASA, Cuiabá, Brazil,* [2]*Faculdade Israelita de Ciências da Saúde Albert Einstein, São Paulo, Brazil,* [3]*Federal University of Mato Grosso, Cuiabá, Brazil,* [4]*DASA, São Paulo, Brazil,* [5]*Exame - DASA, São Paulo, Brazil,* [6]*University of São Paulo School of Medicine, São Paulo, Brazil,* [7]*Federal University of São Paulo – Unifesp, São Paulo, Brazil*

Introduction

The definition of reference range (RR) is a major challenge for clinical laboratories, especially in the Pediatric age group. Although each service should have its own RR, an overall value is used as a reference, disregarding social, regional and economic differences, which may affect the interpretation of results.

Ferritin is a protein involved in cellular iron homeostasis[1], in addition to being an acute phase reactant[2]. In pediatrics is more commonly used as a marker of evaluation of iron stores in the children and adolescents body.

Objective

To define the reference range (RR) of ferritin in healthy children and adolescents aged 1 to 12 years 11 months and 29 days from Cuiabá, capital of Mato Grosso, Brazil.

Methods

This cross-sectional study was approved by the Ethics Committee in Research of the University of São Paulo Medical School-University Hospital (318/11) and the Júlio Müller University Hospital-Federal University of Mato Grosso (947/10). We evaluated 1,994 healthy children and adolescents from 25 day-care centers and 20 municipal schools in the city of Cuiabá-MT.

After applying the inclusion criteria, 1,866 children and adolescents were studied. The RRs were generated through statistical analysis, which included Bartlett's test for homogeneity of variances and the Kruskal-Wallis test. The level of significance for multiple comparisons was adjusted by the Holm-Bonferroni method, which allowed us to group the subjects into 3 age groups. The level of significance was 5% and statistical analyses were performed by Stata. Ferritin was measured by electrochemiluminescence.

Results

The subjects were grouped into 3 different age groups: aged 1-3 years, 4-7 years and 8-12 years. The RRs' superior limit found for each age group were 73 ng/mL for the group aged 1-3 years; 103 ng/mL for the group aged 4-7 years and 138 ng/mL for the group aged 8-12 years.

Conclusion

The values of the parameters evaluated here for these different age groups, can represent decision limits for the Brazilian pediatric population contributing to decrease diagnosis difficulties in our country. Similar studies in different Brazilian regions should be performed.

REFERENCES

1.

Arosio, P., Elia, L. & Poli, M. Ferritin, cellular iron storage and regulation. *IUBMB Life* **69,** 414-422 (2017).

2.

Kell, D. B. & Pretorius, E. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics* **6,** 748-73 (2014).

B-173

Reference Range for HOMA IR in Healthy Children and Adolescents in the City of Cuiabá, Mato Grosso, Brazil

N. Slhessarenko[1], M. Slhessarenko[2], C. J. F. Fontes[3], R. S. Azevedo[4], A. Andriolo[5]. *[1]Federal University of Mato Grosso and ALTA-DASA, Cuiabá, Brazil, [2]Faculdade Israelita de Ciências da Saúde Albert Einstein, São Paulo, Brazil, [3]Federal University of Mato Grosso, Cuiabá, Brazil, [4]University of São Paulo School of Medicine, São Paulo, Brazil, [5]Federal University of São Paulo – Unifesp, São Paulo, Brazil*

Introduction

Insulin resistance (IR) may develop from young age, associated with other cardiometabolic risk factors[1]. Together, these conditions contribute to the occurrence of cardiovascular events in adult life, Brazil's leading cause of death. Identifying and treating cardiovascular risk in childhood may reduce future morbidity and mortality.

Laboratories do not indicate insulin reference intervals for the pediatric population, limiting the interpretation of children and adolescents' results[2].

Objective

To define the reference range (RR) of HOMA-IR in healthy children and adolescents aged 1 to 12 years 11 months and 29 days from Cuiabá, capital of Mato Grosso, Brazil.

Methods

This cross-sectional study was approved by the Ethics Committee in Research of the University of São Paulo Medical School-University Hospital (318/11) and the Júlio Müller University Hospital-Federal University of Mato Grosso (947/10). We evaluated 1,994 healthy children and adolescents from 25 day-care centers and 20 municipal schools in the city of Cuiabá-MT.

After applying the inclusion criteria, 1,866 children and adolescents were studied. For this specific analysis, overweight and obese patients were excluded, resulting in 1696 children and adolescents. The RRs were generated through statistical analysis, which included Bartlett's test for homogeneity of variances and the Kruskal-Wallis test. The level of significance for multiple comparisons was adjusted by the Holm-Bonferroni method, which allowed us to group the subjects into 4 age groups for girls and 3 age groups for boys. The level of significance was 5% and statistical analyses were performed by Stata. HOMA-IR was calculated: ((blood-glucose x 0,0555 x insulin) / 22,5.

Results

The girls were grouped into 4 different age groups: aged 1-5 years, 6-9 years, 10 year and 11 to 12 years. The RRs found for each age group were 0.04-1.74; 0.04-3.06; 0.04-3.70 and 0.22-5.19, respectively. The boys were grouped into 3 different age groups: aged 1-6 years, 7-10 years and 11 to 12 years. The RRs found for each age group were 0.04-1.47; 0.04-2.26 and 0.27-5,14, respectively.

Conclusion

HOMA-IR continuously show an increasing trend with age[3], finding compatible with international studies. This sample can be considered representative of the Brazilian population and its results can be used. Other studies in different regions of the country should be performed.

REFERENCES

1.

Shashaj, B. *et al.* Reference ranges of HOMA-IR in normal-weight and obese young Caucasians. *Acta Diabetol.* **53,** 251-60 (2016).

2.

Ballerini, M. G. *et al.* Insulin level and insulin sensitivity indices among healthy children and adolescents. *Arch. Argent. Pediatr.* **114,** 329-36 (2016).

3.

Peplies, J. *et al.* Percentiles of fasting serum insulin, glucose, HbA1c and HOMA-IR in pre-pubertal normal weight European children from the IDEFICS cohort. *Int. J. Obes. (Lond).* **38 Suppl 2,** S39-47 (2014).

B-174

Comparison of Disclosed Smoking Status to the Presence of Serum Nicotine and Metabolites in Maternal Quadruple Screen Specimens

J. J. H. Hunsaker[1], D. LaGrave[2], W. Murphy[2], H. A. Reichman[2], T. M. Snow[2], G. A. McMillin[3], K. L. Johnson-Davis[3], J. R. Genzen[3]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]ARUP Laboratories, Salt Lake City, UT, [3]University of Utah School of Medicine, Department of Pathology, Salt Lake City, UT*

Background: The Centers for Disease Control and Prevention (CDC) reports that the prevalence of cigarette smoking at any time during pregnancy is 1 in 14 (7.2%; *NCHS Data Brief*, 2018). Along with adverse pregnancy outcomes, cigarette smoking can influence the concentration of several markers used in biochemical maternal serum screening. As such, the College of American Pathologists Laboratory Accreditation Program recently introduced a requirement to solicit current smoking status on both prenatal screening requisition forms (CHM.32350) and reports (CHM.32300). The present study was designed to a) determine the prevalence of disclosed smoking status in our patient population, and b) compare disclosed smoking status with the presence of nicotine metabolites in corresponding serum specimens.

Methods: A de-identified dataset of disclosed smoking status for quadruple (Quad) screens (n=39,625) was obtained using an IRB approved protocol. Residual human serum specimens previously tested for maternal Quad screens (n=271) were also obtained from storage (-20°C) and de-identified according to an IRB-approved protocol. Inclusion criteria for these specimens were a negative Quad screen, singleton pregnancy, and the absence of insulin-dependent diabetes, medications associated with fetal defects, family history of neural tube defects (NTDs), or IVF pregnancy. Specimens were selected to specifically include disclosed smokers (n=71), disclosed non-smokers (n=100), and specimens with unknown smoking status (n=100). *Trans* 3′-hydroxycotinine (3-HCOT), cotinine (COT) and nicotine (NIC) concentrations were measured in these 271 specimens by an in-house developed quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. COT concentrations were used to categorize patients as probable smokers (>10 ng/mL), passively exposed or infrequent smokers (2-10 ng/mL), or not exposed (<2 ng/mL).

Results: The retrospective data analysis of 39,625 consecutive Quad screens revealed that 24,611 had reported a status related to smoking or nonsmoking. Of these, 1,783 (7.2%) were disclosed smokers. The 271 retrieved specimens used in subsequent studies represented diverse ages (15-51 yrs old), ethnicities (Asian, n=3; Black, n=60; Caucasian, n=93; Hispanic, n=19; Native American, n=4; Non-Black, n=85; Unknown, n=7), maternal weights (94 – 338 pounds), and U.S. states (n=23). Mean concentrations of measured 3-HCOT, COT, and NIC in the patient groups were as follows (mean ± SD, range): disclosed smokers, 3-HCOT (49.2 ± 44.3, 0-223), COT (80.6 ± 64.4, 0-235), NIC (4.9 ± 5.8, 0-22); disclosed non-smokers, 3-HCOT (2.0 ± 9.5, 0-79), COT (4.2 ± 21.5, 0-172), NIC (0.1 ± 0.8, 0-6); unknown, 3-HCOT (6.4 ± 23.8, 0-186), COT (12.6 ± 42.9, 0-226), NIC (0.5 ± 2.0, 0-13). Using COT general cutoff values, these patients were further categorized as a) probable smokers, b) passively exposed and/or infrequent smokers, or c) not exposed: disclosed smokers [a) 83.1%, b) 2.8%, c) 14.1%]; disclosed non-smokers [a) 6.0%, b) 3.0%, c) 91.0%]; unknown [a) 13.0%, b) 4.0%, c) 83.0%].

Conclusions: Disclosed smoking rates in our patient population are similar to those previously reported by the CDC. Active smoking rates may be lower in disclosed smokers and higher in disclosed non-smokers and those with unknown smoking status.

B-175

Minimum Sample Volume Requirement and Performance Evaluation of the Siemens Atellica CH and IM Analyzer for Paediatric Patients

N. Sahabdeen, T. Shi Yan, Y. Chun Haw, A. Pajarillaga, K. Yah Hwee, W. Wei Xuan, S. Saw, S. Sethi. *National University of Singapore, Singapore, Singapore*

Background: The aim of this study was to evaluate minimum sample volumes and assay performance of commonly requested tests for our paediatric patients. Testing was performed on Atellica™ Chemistry(CH) and Immunoassay(IM) Analyzer (Siemens Healthineers, Tarrytown, USA) for Potassium, Sodium, Urea and Creatinine as a Renal Panel (RP) as well as Free Thyroxine (FT4) and Thyroid-Stimulating Hormone (TSH) as a Thyroid (TY) panel.

Methods: Becton Dickinson (New Jersey, USA) microtainer green lithium heparin and yellow serum-separator (SST) tubes with Capillary Tube Holder and 1ml Tubetop sample cups (provided by Siemens) were used to determine minimum sample

volume required. Varying volumes between 35-500ul of pooled residual plasma were measured and added to each container type per test configuration. Verification of precision used four patient plasma pools of different concentrations across the analytical measuring range (AMR) in singlecate for ten days and four replicates for one day. Method comparison studies were performed using 120 previously tested plasma samples from AU5800/Dxi (Beckman Coulter, Brea, USA). Six levels of linearity material in replicates of each sample level were tested across AMR. **Results:** The lithium heparin microtainer tube requires 500ul of plasma for RP, 300ul of plasma for TY and 700ul for simultaneous testing. Yellow SST microtainer tubes requires 300ul of plasma for RP, 200ul of plasma for TY and 300ul for simultaneous testing while the 1ml Tube-top sample cup requires only 80ul of plasma for RP, 120ul of plasma for TY and 200ul for simultaneous testing. Potassium, Sodium, Urea, Creatinine, FT4 and TSH within-run CVs were 0.4-0.9%, 0.5-1.0%, 0.0-1.9%, 0.2-1.4%, 2.1-5.1% and 1.4-6.5% respectively. Potassium, Sodium, Urea, Creatinine, FT4 and TSH-UL total imprecision CVs were 0.6-1.0%, 0.5-2.1%, 1.3-3.2%, 0.4-1.7%, 3.9-7.3% and 2.7-4.6% respectively. Linear regression slope and correlation coefficient (r) for the tested analytes were Potassium 0.987, r=0.997; Sodium 0.942, r=0.979; Urea 1.051, r=0.997; Creatinine 0.967, r=1.000; FT4 0.796, r=0.908 and TSH-UL 0.952, r=0.994. Percentage recovery for linearity study for Potassium, Sodium, Urea, Creatinine, and TSH were between 99.5-101.3%, 99.7-101.0%, 104.1-105.8%, 98.1-100.9%, and 90.5-106.1%respectively. **Conclusion:** Many laboratories would like a solution to automate paediatric testing. A good option is provided here with simultaneous testing able to report all results with a lower sample volume. However when volume is a significant limiting factor, the 1ml Tube-top sample cup is still superior to direct sampling from microtainers. The assays tested on the Siemens Atellica® CH and IM Analyzer demonstrated good agreement to our current analyser and precision results were consistent with manufacturer's claims.

B-176

Standardization of Neonatal Bilirubin Testing and the Lack of a Primary Reference Material

S. Lo[1], L. Hubbard[2], M. Koranda[2], B. Doumas[1]. *[1]Medical College of Wisconsin, Milwaukee, WI, [2]Children's Hospital of Wisconsin, Milwaukee, WI*

Objective: The lack of a primary reference material for bilirubin has hampered the ability reference measurement laboratories to maintain traceability for bilirubin testing. To circumvent this issue, our laboratory created a secondary reference material. We demonstrate the consistency of this material for use with the Doumas reference method procedure and reviewed College of American Pathologists (CAP) NB-Survey proficiency testing results to assess if current neonatal bilirubin testing has been affected.

Materials and Methods: Stock solutions of unconjugated bilirubin (Bu) were prepared using NIST SRM 916, NIST candidate reference material, and Lee Biosolutions (St. Louis, MO). Human serum-based controls were also made and the Doumas method (Clin Chem 1999, 45:1255-60) used to create calibration curves and quantitate samples. CAP NB-Surveys from 2004 thru 2017 were reviewed.

Results: Stability of the calibration curve used in the reference method over 14 years indicate a molar absorptivity coefficient and a slope %CV of 0.5%. The %CV for human serum-based controls from two time periods at two concentrations one using SRM916 and the other using a secondary material are similar. SRM 916 mean=4.77 mg/dL; %CV=0.8 and mean=17.74 mg/dL; %CV=0.9 versus secondary material mean=5.17 mg/dL; %CV=0.8 and mean 18.31 mg/dL; %CV=0.4. CAP NB-Survey peer group means from 2004 and 2017 were compared to reference method targets. In 2004, 9 of 22 peer group methods were within 10% of the target value compared to 25 of 26 in 2017.

Conclusions: Secondary reference materials used with the reference measurement procedure can be used to maintain traceability in bilirubin assays. Review of CAP NB-Survey results suggest that the overall performance of neonatal bilirubin testing has improved since 2004.

B-177

Validation of the Lumipulse® G Anti-Müllerian Hormone (AMH) Assay for Quantitative Determination of AMH in Human Serum and Plasma

N. Benina[1], K. Falcone[1], S. Raju[1], S. Marcoon[1], J. Latham[1], K. Le[1], A. Calabro[1], M. Urbanski[1], S. Syjjongtian[1], U. Dahlen[2], C. Fermer[2], G. Lambert-Messerlian[3], E. Eklund[3], K. Donaldson[4], P. Wheeler[4], J. Genzen[5], J. Hunsaker[5], R. Radwan[1], S. Dolan[1], D. Dickson[1], C. J. Traynham[1]. *[1]Fujirebio Diagnostics Inc, Malvern, PA, [2]Fujirebio Diagnostics AB, Gothenburg, Sweden, [3]Women and Infant's Hospital in Rhode Island, Providence, RI, [4]Prescient Medicine, Hummelstown, PA, [5]ARUP, Salt Lake City, UT*

Introduction: Anti-Müllerian hormone (AMH) is a glycoprotein from the TGF-β family that is produced by granulosa cells in ovarian follicles. AMH levels represent the number of small follicles entering their life cycle growth phase. Thus, *in vitro* determination of AMH can be used to aid in the evaluation of ovarian reserve**. Methods:** The Lumipulse G AMH assay is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the *in vitro* quantitative determination of AMH in human serum and plasma on the LUMMIPULSE G System. Within specimens, AMH specifically binds to anti-AMH monoclonal antibody (mouse) coated on particles with antigen-antibody immunocomplexes subsequently formed. After completion of a washing step, alkaline phosphatase (ALP; calf)-labeled anti-AMH monoclonal antibody (mouse) specifically binds to AMH immunocomplexes on particles with additional immunocomplexes formed. The amount of AMH within specimens is derived when substrate solution is added to the reaction mixture with luminescence quantified when AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt) is dephosphorylated by ALP. All verification and validation studies were performed according to respective CLSI guidelines. **Results:** The Limit of Blank, Limit of Detection and Limit of Quantitation expressed through functional sensitivity of the Lumipulse G AMH assay were 0.005, 0.011, and 0.022 ng/mL, respectively. The Lumipulse G AMH assay demonstrated linearity in the range from 0.024 to 27.159 ng/mL. No high-dose hook effect was observed in samples containing up to ~1,000 ng/mL of AMH. A twenty-day precision study of 5 human serum-based panels assayed in duplicate at two separate times of the day (n = 80 for each panel) demonstrated within-laboratory (total) precision of ≤ 2.1%. Spike-recovery testing demonstrated that the Lumipulse G AMH assay is capable of recovering known concentrations of supplemental analyte (% recovery range, 95-107%). Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 12 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, biotin, ascorbic acid, human anti-mouse antibody, human serum albumin, chyle, immunoglobulin G, alkaline phosphatase, rheumatoid factor) and 41 commonly used therapeutic drugs. Cross- reactivity of the Lumipulse G AMH assay with other substances (Human Follicle Stimulating Hormone (500 mIU/mL), Human Luteinizing Hormone (500 mIU/mL), Inhibin A (100 ng/mL), Activin A (100 ng/mL), and TGF β-1(65 ng/mL), respectively) that are similar in structure to AMH demonstrated no cross-reactivity. A freeze-thaw study revealed that no more than 2 freeze-thaws should be performed in samples collected in the following tubes: Red top serum, SST serum, K2EDTA plasma, and Lithium Heparin plasma tubes. Further, a long-term sample stability study revealed that AMH concentrations are stable up to ~ 20 months. A method comparison of Lumipulse G AMH with a FDA-cleared predicate device was analyzed using weighted Deming regression. In the 137 tested specimens (concentrations ranged from 0.090 to 21.168 ng/mL), the slope, y-intercept, and correlation coefficient (r) were 1.039, 0.0097, and 0.997, respectively. **Conclusions:** The data demonstrate that the Lumipulse G AMH assay on the automated LUMIPULSE G1200 System is precise, sensitive, and accurate for routine quantitative determination of AMH in serum and plasma specimens.

B-178

Optimization of the Newborn Screening Algorithm for Cystic Fibrosis in the State of New Jersey

M. O. Carayannopoulos[1], M. Schachter[2], K. Dynak[2], E. McElroy Barker[1]. *[1]Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, [2]New Jersey Department of Health, Ewing, NJ*

Background: The state of New Jersey recently implemented a plan to improve its screening algorithm for Cystic Fibrosis (CF). This optimization project was prompted by the fact that the existing algorithm did not meet recommended national standards. The two-tiered screening protocol was suboptimal both based on the insensitivity of the immunoreactive trypsinogen (IRT) cut-off and the number of CFTR variants (ΔF508 only) screened. It is estimated that 5 children/year were not diagnosed at birth

due to inadequacies in the algorithm. The optimization plan consists of two distinct phases. In phase one, the IRT cut-off will be lowered which will increase the sensitivity of the primary screen and can be implemented with minimal resources. Next, to improve the sensitivity and specificity of the 2nd tier screen, a molecular assay that detects 139 CFTR variants will be introduced. The impact of each change will be analyzed independently. Once fully implemented, the screening algorithm for CF in New Jersey will exceed national standards. **Methods:** The IRT cut-off was lowered from 90 ng/ml to 70 ng/ml in April 2018. Any specimen with an IRT above the cut-off was reflexed to analysis for the presence of ΔF508. Infants with an elevated IRT and 1 or 2 copies of the variant were referred to a specialist for confirmatory testing. Additionally, due to the insensitivity of the 2nd tier screen, infants with only an elevated IRT (i.e. no copies of ΔF508) were also referred to follow-up. This was an enhancement from the previous algorithm, in which infants with isolated elevations of IRT were reported as "within acceptable limits." **Results:** The impact of lowering the IRT cut-off was assessed. From April 2, 2018 - January 31, 2019, at total of 81,322 infants were screened. Of these 1,138 (1.4%) had an IRT ≥ 70ng/ml. Within this population, 775 had an IRT of between 70 ng/ml - 90ng/ml and represents the cohort of infants impacted by the change in IRT cut-off. Within this population, 763 infants had zero copies of ΔF508 and 12 infants had 1 copy of ΔF508. Of these infants, we have one confirmed case of CF and one diagnosed with CFTR-related metabolic syndrome. **Conclusion:** Phase one of the optimization plan to improve the newborn screening algorithm in the state of New Jersey is complete and has successfully diagnosed two infants who would have otherwise been missed. These data emphasize the importance of ongoing evaluation of screening cut-offs. Once both phases of the optimization plan have been implemented, we are confident that screening for CF in the state of New Jersey will exceed national standards resulting in fewer missed cases during the newborn period and better long-term outcomes.

B-179

Trimester-Specific Reference Intervals for Folate, Vitamin B12, and Active B12 in Pregnancy

J. Noguez, L. Maurer. *University Hospitals Cleveland Medical Center, Cleveland, OH*

Background: Anemia in pregnancy is associated with poor outcomes such as increased risk for pre-term delivery, low birth weight, and blood loss during labor. Therefore the timely diagnosis and treatment of prenatal anemia is critical. Pregnancy-specific or trimester-specific reference intervals may be required due to the numerous physiological changes that occur to accommodate the needs of a growing fetus. We aimed to identify trimester-specific normal ranges for several serum biomarkers to facilitate the diagnosis and treatment of prenatal macrocytic anemia.

Methods: A total of 120 pregnant women with normal blood hemoglobin levels (WHO/ACOG definitions) were tested during the first (n = 40), second (n = 40), and third trimesters (n = 40) for serum levels of folate, vitamin B12, and active B12. First trimester was defined as gestational week 1-12, second trimester as week 13-26, and third trimester as week 27-40. Forty age-matched, non-pregnant women with normal blood hemoglobin levels were also tests as a control group. Folate and vitamin B12 levels were determined using the Siemens Centaur XP immunoassay analyzer and active B12 measurements were performed on the Abbott Architect i1000s. Descriptive statistics (mean, median, and standard deviation) were determined for each group and reference intervals were defined as the 2.5th and 97.5th percentiles. Two-tailed t-tests were used to determine statistically significant differences between the non-pregnant control group and pregnant patients in each trimester.

Results: Mean age ± standard deviation of study patients was 29 ± 6 years. The mean values ± standard deviation in the control group, first, second and third trimesters, respectively, were as follows: folate 12.1 ± 5.2, 16.1 ± 5.7, 17.3 ± 5.7, 14.4 ± 6.7 ng/mL; vitamin B12 517 ± 208, 395 ± 184, 362 ± 128, 341 ± 111 pg/mL; and active B12 81.7 ± 35.2, 78.3 ± 33.0, 78.6 ± 37.6, 90.1 ± 46.6 pg/mL. Statistically significant differences were observed between the non-pregnant control group and each trimester of pregnancy for vitamin B12. No statistically significant differences were observed between the non-pregnant control group and the pregnant patients for folate and active B12.

Conclusion: This study defines trimester-specific normal ranges for folate, vitamin B12, and active B12 in pregnancy. The data demonstrates that a single reference interval of > 5 ng/mL for folate and 21.0- 215.0 pg/mL for active B12 can be used for both pregnant and non-pregnant females in the age range 16- 43 years old. Statistically significant differences in vitamin B12 levels throughout pregnancy warrants trimester-specific reference intervals of 203-787, 157-581, and 206-616 for the first ($P < 0.015$), second ($P < 0.0001$), and third trimesters ($P < 0.0001$) respectively.

B-180

Infant Skin Tone Affects the Relationship between Transcutaneous and Total Serum Bilirubin

J. L. Robinson, J. L. Gifford, A. A. Venner. *Alberta Public Laboratories, Calgary, AB, Canada*

Background: The ontogeny of liver enzymes is such that newborns frequently experience hyperbilirubinemia and jaundice. Neonatal jaundice usually resolves naturally, but prolonged hyperbilirubinemia can lead to permanent encephalopathy and kernicterus. Fortunately, kernicterus is preventable by phototherapy that photoisomerizes and oxidizes bilirubin for biliary and renal elimination. The Neonatal Jaundice Screening Program in our health region screens all infants for hyperbilirubinemia using either total serum bilirubin (TSB) or transcutaneous bilirubin (TcB). TcB is a non-invasive method to predict TSB using a bilirubinometer, which flashes light into the skin and measures the return of light at specific wavelengths. TcB values are interpreted using a locally developed nomogram that directs infant follow-up, potentially including TSB testing and phototherapy.

Objective: Determine the predictive value of TcB for TSB concentrations and determine whether this relationship is affected by infant skin tone using two commercial bilirubinometers.

Methods: TcB was measured by Public Health nurses in Southern Alberta, Canada from 2014-2018 on 2,937 newborns. For each infant, there was an objective skin tone assessment (i.e. light, medium, or dark), a TcB result, and a corresponding TSB result that was collected within one hour of the TcB assessment. TcB was measured using Drager JM-105 and JM-103 meters, and TSB was quantified by clinical laboratory chemistry analyzer (Roche Diagnostics cobas c 501 or Ortho Clinical Diagnostics Vitros 350). TcB and TSB values were compared by linear regression and bias plots for each of light, medium, and dark skin toned newborns.

Results: TcB values of infants with light (N=1,424) and medium (N=1,156) skin tone were positively biased *versus* TSB results until TSB values reach 256 µmol/L (95% CI: 253-259 µmol/L) and 270 umol/L (95% CI: 268-273 µmol/L) for light toned and medium tones infants, respectively. The TcB meters were negatively biased in infants with light and medium skin tone *versus* TSB. In contrast, TcB values from dark toned (N=359) infants showed a robust positive bias relative to all TSB values, although the regression line intercepted at 317 µmol/L (95% CI: 305-333 µmol/L). Median absolute and relative bias' were significantly different among skin tones (p<0.05). Moreover, the slope of each TcB bias plot were different between the three skin tones (p<0.05).

Conclusion: TcB meters demonstrated a differential bias based on skin tone. This may require specific nomograms for an increasingly diverse Southern Alberta population, and requires further analytical and clinical investigation.

B-181

Values of Vitamin A in the Pediatric Population in Brazil in the Evaluation of Malnutrition

D. V. Gomes, S. T.S.P., F. M.D, S. P.C., C. G.A., S. Natasha. *DASA, Rio de Janeiro, Brazil*

Background: Vitamin A (retinol) is a fat soluble micronutrient, whose evaluation helps to monitor nutritional deficiencies. A reduction of vitamin A is expected, along with other liposoluble proteins, in cases of malnutrition. **Objective:** Our objective is to report vitamin A values in the pediatric population in Brazil in the absence of gastrointestinal diseases, considering the nutritional difference between the northeastern and north regions with higher malnutrition index compared to the south and southeast, as well as greater malnutrition in children under 5 years. **Methods:** A total of 9905 patients without reports of vitamin replacement use and without reports of malabsorption syndrome were separated from the laboratory routine during the year 2018. The sample was divided into four groups: Group A (0 to 1 year), Group B (2 to 11 years), Group C 12 to 16 years) and Group D (17 to 19 years). Vitamin A dosages were performed by the High Performance Liquid Chromatography method. The number of Group A samples = 462, B = 4580, C = 3201, D = 1662. The samples of whole blood were of children and adolescents of four Brazilian regions: Southeast, Northeast, Center-West and South. **Results**: Similarly to the data obtained by Caliper (Canadian Laboratory for Pediatric Reference Intervals), we obtained an increasing value of vitamin A with age, Pearson's correlation=0,17. The difference between the regions studied was assessed by the Test T, with a confidence level of 95%, comparing north and northeast regions with south and southeast, in all age groups. We found no difference between the means of vitamin A in groups A and B, p=0.08 and p=0.44, respectively. However, a difference was observed between groups C and D, p=0.03 in

both groups, with a higher mean in the Southeast and South regions. **Conclusion:** We did not observe differences between the regions with the highest malnutrition index and those with the lowest index for mean vitamin A for the lower age groups, below 11 years, which is not in agreement with the population studies carried out by health agencies in Brazil. The difference was seen only in children and adolescents above 11 years of age. Non-compliance may be due to the higher socioeconomic level of children with access to exams in analysis laboratories

Wednesday, August 7, 2019

Poster Session: 9:30 AM - 5:00 PM
Critical and Point-of-Care Testing

B-182

Accuracy of Various Estimated Creatinine Clearance Equations in Estimating Glomerular Filtration Rate in Indians

R. K. Nigam. *Rajiv Gandhi College, Barkatullah University, Bhopal, India*

Background: The aim of this study was to compare the efficacy of GFR derived from various estimated creatinine clearance methods like Jelliffe, Cockcroft and Gault, and 4MDRD equations as compared to measured glomerular filtration rate (GFR) with in Indians .

Methods: We enrolled 80 patients in the study. GFR was determined by technetium-99m diethyl triamine penta-acetic acid (Tc99mDTPA) clearance . Height, body weight and serum creatinine were measured, and GFR and creatinine clearance (CrCl) estimates calculated by various equations. Spearemans correlation was used to assess relationships between measured GFR (Tc99mDTPA clearance) and estimated clearances using the three formulae. Difference between the measured GFR and estimated clearances compared with measured GFR were examined to determine whether prediction error was independent from measurement magnitude. Analyses of differences were used to determine bias and precision. Bias was assessed by mean percentage error (MPE), calculated as the percentage difference between the estimated clearances for each formula and measured GFR. A positive bias indicates overestimation of GFR, and a negative bias indicates underestimation. Relationships were also assessed by gender and varying levels of renal function: GFR <60 ml / min, and GFR >60 ml/ min.

Results: The mean measured GFR was 77.2 ml / min (range 17 to 152 ml / min). The mean bias (mean percentage error) was -4.9, -10.3 and -1.57% respectively for the, Jelliffe , Cockcroft and Gault, and 4MDRD formulas, respectively. The 4 MDRD formula slightly overestimates the GFR in patients having GFR less than 60ml/ min, where as,it underestimates for GFR more than 60ml / min.

Conclusion: In Indians 4 MDRD equation of estimated creatinine clearance seems to be most accurate in estimating GFR .

B-183

Comparison of Two Point of Care Analyzers: Accu-ChekPerforma II Roche and Nova Phox Ultra, In The Measurement of Glycemia in Whole Blood

A. Salinas, V. Ricci, M. Menises, P. Domecq. *Laboratorio Domecq & Lafage, Hospital Alemán, Buenos Aires, Argentina*

Background: The recognition of both hypo and hyper glycemia is important for the decision making by the health team. The Point-of-Care Testing (POCT) allows the measurement of glucose in a fast and simple way. In our center, we have two kind of POCT: Accu-ChekPerforma II Roche (method: glucose dehydrogenase), used by doctors and nurses and the Nova Phox Ultra (method: glucose oxidase) used inside the laboratory. It should be noted that the results obtained with both analyzers should be comparable, so that medical decision is not affected. The aim of the study was to assess the comparability of glucose measurement between both analyzers available in our center.

Methods: Prospective study in which 78 samples, randomly selected, of whole blood obtained in heparinized syringe were analyzed in parallel in both analyzers. The results were assessed with the Bland-Altman method, Deming regression and Clinical and Laboratory Standard Institute (CLSI) protocol EP15 A2.

Results: The mean obtained with the Accu-Chek was 129±50 mg/dl (range: 66 to 289) and the mean with the Nova Phox Ultra was 134±51 mg/dl (range: 76 to 312). Deming regression demonstrated a slope of 1,014 (95% CI 0,962 to 1,066) and intercept of 3,0 mg/dl (95% CI -3,3 to 9,4), which indicates that it does not exist a statistically significant difference between both methods. By Bland-Altman method, a positive bias of 4,81 (95% CI 2,88 to 6,74) was observed in favor of Nova Phox Ultra. To determine if this bias was greater than the one accepted by the laboratory, the CLSI protocol EP15 A2 was carried out, taking into account the quality requisition stablished by Clinical Laboratory Improvement Amendments (CLIA) of 10%. Through this analysis, it was observed that the bias did not exceed the 50% of the total error accepted.

Conclusion: The estimated bias was lower than the allowable bias. Therefore, glucose results between Accu-Chek Performa II Roche and Nova Phox Ultra are interchangeable. This ensures no impact on patient care when using alternatively both analyzers. Furthermore, bearing in mind, that the Nova Phox Ultra is the instrument inside the laboratory which usually undergoes quality controls, both internal and external, the demonstrated comparability may allow the design of a strategy for the assessment of performance of those equipment used exclusively for the bedside assistance.

B-184

Can Ethylene Glycol Metabolites be Detected Using a Point of Care Lactate Meter to Assess the Lactate Gap? A Case Study

J. Macpherson, D. Ethier, F. Wu, A. W. Lyon, M. E. Lyon. *University of Saskatchewan, Saskatoon, SK, Canada*

Background: EG poisoning typically manifests with metabolic acidosis and an elevated anion gap. Glycolate and glyoxylate, the toxic metabolites of EG, can cause an artefactual increase in lactate measured by some enzymatic methods. A lactate gap is a clinically significant discrepancy in lactate results obtained with different methods. The definitive diagnosis of EG poisoning is made by the detection of EG in the serum, but the presence of a Lactate Gap may suggest the presence of EG metabolites when the cause of an anion gap is unknown.

Objective: To investigate potential use of the Nova® Statstrip lactate meter and Radiometer® ABL 837 blood gas analyzer in determining the lactate gap in specimens from a patient presenting with EG ingestion.

Methods: Serum EG concentrations were measured using a gas liquid chromatographic (GLC) method developed in the clinical laboratory at Royal University Hospital. Venous blood gas specimens were analyzed using a Radiometer ABL837 blood gas analyzer. These samples were also measured using the Nova StatStrip lactate meter.

Case Report: A 17 year old male presented to emergency medicine after intentional ingestion of ~500ml of antifreeze. They reported blurred vision and dizziness; vital signs were normal and the Glasgow Coma Score was 15. Table 1 illustrates the EG, pH, anion gap, lactate, and lactate gap results. The patient was treated with Fomepizole, and hemodialysis over 6 hours. The patient improved clinically and was discharged two days later.

Conclusions: The patient presented with an elevated anion gap and lactate gap that suggests that EG metabolism had occurred at presentation and both resolved following therapy. The Nova Statstrip lactate meter method did not appear to detect EG metabolites. Calculation of a lactate gap using the Nova Statstrip lactate meter and Radiometer ABL837 has potential to detect EG metabolites in exposed patients.

Table 1: EG, pH, anion gap, lactate and lactate gap results

Time	EG (mmol/L)	pH	Anion Gap (mmol/L)	ABL837 Lactate (mmol/L)	Nova Lactate (mmol/L)	Lactate Gap (mmol/L)
04:00	5.8	7.25	21	18	4.3	13.7
04:28	7.1	7.25	17	17	3.9	13.1
10:30	1.7	7.38	8	2.5	2.0	0.5

B-185

Performance Evaluation of Atellica UAS800 at a University Hospital

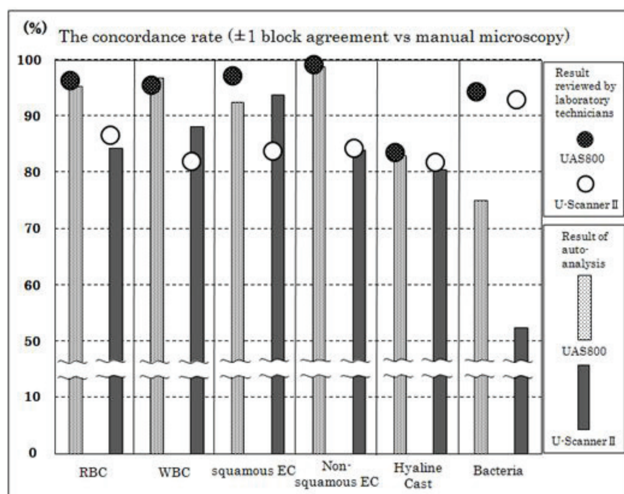
C. Yokoyama[1], C. Negishi[1], S. Watabe[1], T. Nanmoku[1], Y. Kawakami[2]. [1]*Department of Laboratory Medicine, University of Tsukuba Hospital, Tsukuba, Japan,* [2]*Department of Clinical Pathology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan*

Background: Microscopic analysis of urine samples, known as urinary sediment examination, is a commonly performed test in clinical laboratories that is used to screen for several diseases. However, the manual microscopy traditionally involved in urinary sediment examination is time-consuming. Currently, the automated urine sediment analysis methods available in Japan fall into 2 categories, namely, flow cytometry and image processing. Of this latter category, the UAS800 (Siemens) performs its image analysis after centrifuging the sediment and, with its dual-focusing system, produces clear images. The objective of this study was to verify the analytical performance of the UAS800.

Methods: In this study we used 424 fresh urine samples. We compared 2 automated urine sediment analyzers that both utilize the image processing method: the UAS800 and the U-SCANNERII (Toyobo). We also compared manual urine microscopic analysis using similar evaluation parameters. Furthermore, we evaluated the results of the auto-analysis of the 2 machines by comparing it with the manual-analysis performed by qualified laboratory technicians. The parameters we evaluated were the rates of agreement for each item.

Results: For RBC, the concordance rate between UAS800 (auto-analysis) and manual microscopy was 62.7% (exact agreement) and 95.4% (±1 block agreement), while the U-SCANNERII was 45.5% (exact agreement) and 84.3% (±1 block agreement) . Similarly, for RBC, the concordance rate between UAS800 (reviewed by laboratory technicians) and Manual microscopy was 63.7% and 95.8%, while U-SCANNERII was 47.6% and 85.4%. Concordance rates for the other items are shown in Figure 1.

Conclusion: The UAS800 showed satisfactory concordance with the manual microscopy method in auto-analysis and was improved with manual review by laboratory technicians. Thus, the UAS800 will contribute towards improving the efficiency of urinary sediment analysis.



B-186

Evaluation of the Atellica UAS800 Urine Sediment Analyzer

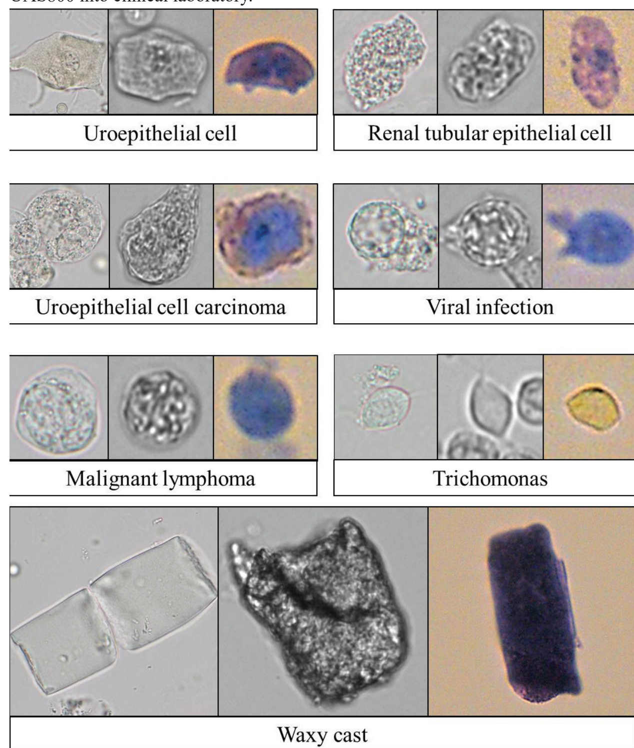
Y. Morita[1], K. Shukuya[2], M. Tanaka[1], T. Hisasue[1], M. Sato[1], Y. Ono[1], T. Sato[1], M. Kurano[3], Y. Yatomi[3]. *[1]Department of Clinical Laboratory, The University of Tokyo Hospital, Tokyo, Japan, [2]Department of Medical Technology and Sciences, International University of Health and Welfare, Fukuoka, Japan, [3]Department of Clinical Laboratory Medicine, The University of Tokyo, Tokyo, Japan*

Background: Urine sediment test is usually performed by manual microscopy, however this task requires too much time and effort. Recently, some instruments based on either digital imaging or flow cytometry have been developed to automate the urine sediment test. These instruments could reduce the number of the cases which require the examination with manual microscopy. In the present study, we validated Atellica UAS800 urine sediment analyzer (Siemens HealthCare Diagnostics) to investigate whether this instrument would be equipped with the basic performance enough to be introduced into clinical practice.

Methods: 541 residual urine specimens after clinical examination in the University of Tokyo Hospital were analyzed using three automated urine analyzers: Atellica UAS800 based on digital imaging with Full field view images, UF-1000i based on flow cytometry (Sysmex corporation), and USCANNER(E) (TOYOBO), which is based on digital imaging. This study was approved by the Institutional Research Ethics Committee of the Faculty of Medicine, the University of Tokyo.

Result: UAS800 had good concordance rates within 1 group agreement with microscopic examination, for red blood cell (RBC) (97.8%), white blood cell (WBC) (95.1%), and squamous epithelial cells (EPI) (94.8%). The counts of RBC, WBC, and EPI measured with UAS800 were moderately correlated with those measured with UF-1000i (r=0.922, y=1.013x-1.738, r=0.878, y=0.451x+7.896, and r=0.808, y=1.506x-0.524, respectively), however WBC counts measured with UAS800 tended to be lower, while EPI tended to be higher. Compared with USCANNER(E), UAS800 provided clearer images even without cell staining (Figure). Regarding renal tubular cells and casts, the performance of UAS800 was insufficient and the proof with manual microscopy was required.

Conclusion: UAS800 provided reliable results for RBC, WBC, and EPI, while the performance in classification of renal tubular cells and casts was not enough. Therefore, the combination with manual microscopic examination is required to introduce UAS800 into clinical laboratory.



Manual microscopy (left), UAS800 (middle), USCANNER(E) (right)

B-187

Clinical Evaluation of Pleural Fluid pH Measurements on the GEM Premier 5000

K. Manley[1], J. Toffaletti[2], E. Handel[2]. *[1]Instrumentation Laboratory, Bedford, MA, [2]Duke University Hospital, Durham, NC*

Pleural effusions are abnormal accumulations of fluid in the pleural space between tissues lining the lungs and chest cavity, with pH measured in exudative effusions to guide therapeutic decisions. Pleural fluids with pH less than 7.30 are potentially malignant effusions. The current reportable range for pleural fluid pH is 7.00-7.50 with medical decision levels defined at 7.20 and 7.30.

With the American College of Chest Physicians recommending the use of blood gas analyzers to measure pleural fluid pH, we evaluated the GEM Premier 5000 (Instrumentation Laboratory, Bedford, MA) for measuring pleural fluid pH at Duke University Hospital. All testing was done by hospital point of care users who tested 52 pleural fluids collected from patients with a variety of pleural effusions and 10 contrived samples from pooled pleural fluids in order to span the entire reportable range. A Bland-Altman analysis gave a mean difference to the Radiometer® ABL835 FLEX (Radiometer Medical, Denmark) of -0.006 (SD of diffs 0.032). For samples near the medical decision levels, 3 had pH from 7.17 to 7.19 and 10 had pH from 7.26 to 7.35 and all were within 0.04 of the comparative method. Of the 62 comparisons, 58 were within the allowable error of 0.06, with the other 4 all having pH >7.44, well above the medical decision levels.

Along with good correlation to the laboratory-based instrument, the GEM 5000 has improved operational simplicity and quality management. Specifically, the GEM 5000 utilizes a PAK that contains all components required for testing, eliminating user maintenance or troubleshooting. The quality management system on the GEM 5000 provides continuous and automatic quality checks before, during, and after each sample analysis. The GEM 5000 is an easy to operate and analytically reliable point of care solution for pleural fluid pH testing.

Table: Method Comparison of Pleural Fluid pH between GEM 5000 and ABL 835

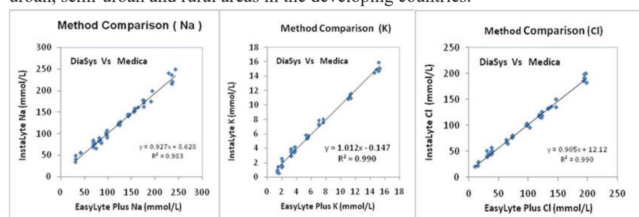
Pleural Fluid pH Range	n	GEM 5000 vs. ABL 835		# within Total Allowable Error (±0.06)	Slope	r value
		Mean Difference	SD of Differences			
7.17 – 7.19	3	0.03	0.00	3	---	---
7.26 – 7.35	10	-0.02	0.014	10	---	---
7.00 – 7.50	62	-0.006	0.032	58	0.91	0.972

B-188

A Novel All-in-One Disposable Cartridge Based Truly Modular Electrolyte Analyzer

V. MATHUR. *DIASYS DIAGNOSTICS INDIA PVT. LTD, MUMBAI, India*

Background: Measurement of electrolyte tests is common in labs worldwide for screening of illness or diseases. Most of the electrolyte analyzers have sensors, reagents, sampler, pump tubing, valve, separate that are expensive and require heavy maintenance (weekly, monthly, quarterly etc).. In order to provide healthcare diagnostics to ALL, there is a need for an All-in-one Universal Disposable Cartridge that contains sensor, reagents, sampler, valve, and pump tubing, in one single package that is affordable (cost per test), reliable, easy-to-use, is truly modular and maintenance-free. DiaSys India has developed World's First, Patent Pending, Versatile, Long life, All-in-One Disposable Cartridge based Electrolyte Analyzer (QDxInstaLyte) that is capable of measuring Na,K,Cl,Li,Ca,pH in whole blood/plasma/serum/qc/csf and diluted urine samples. Our proposed methodology utilizes an innovative, long life ISE sensors to provide a sensitive and accurate result in 2 min with just 75-130 uL of sample volume. **Methods:** We have evaluated the All-in-One Disposable NaKCl Cartridge for linearity, precision and cartridge stability. Method comparison was done against Medica EasyLyte Plus Na/K/Cl Analyzer, with N = 80 samples in 2 weeks. Precision study was done using modified CLSI guidelines with N = 10 samples. Accelerated stability testing was done at 45°C for 4 weeks at 0, 7, 14, 21, 28 days respectively. **Results:** Data analysis indicates that the CV for within-run precision for Na is < 1%, for K is <2%, and for Cl is <2% with R2 > 0.98 for Method Comparison. Further, the All-in-One Disposable Cartridge is stable up to 12-18 months at 2-25°C storage temperature based on preliminary extrapolated data. **Conclusion:** The Developed Technology Platform of All-in-One Disposable based QDxInstaLyte is reliable, is truly modular and maintenance-free. Hence, it can be easily adapted for low cost, accurate and rapid measurement of electrolyte tests in low resource settings such as in urban, semi-urban and rural areas in the developing countries.



B-189

Clinical Evaluation of the GEM® Premier™ 5000 at Albert Einstein Israelita Hospital, São Paulo, Brazil

C. dos Santos Ferreira[1], A. Caschera Leme[1], F. Donde Menegat[1], J. Conant[2], N. Raymond[2], J. Cervera[2]. [1]*Hospital Israelita Albert Einstein, Morumbi, São Paulo, Brazil,* [2]*Instrumentation Laboratory, Bedford, MA*

Background: The GEM Premier 5000 is a blood gas analyzer for rapid analysis of whole blood samples at the point of care or in a central laboratory. This analyzer contains an all-in-one GEM PAK to provide quantitative measurements of pH, pCO2, pO2, sodium, potassium, chloride, ionized calcium, glucose, lactate, hematocrit, total bilirubin and CO-Oximetry (tHb, O2Hb, COHb, MetHb, HHb, sO2) parameters. These measurements (and derived parameters) aid in the diagnosis of a patient's acid/base status, electrolyte and metabolite balance and oxygen delivery capacity. **Methods:** Clinical and usability performance of the GEM Premier 5000 was evaluated at Albert Einstein Hospital compared to the ABL 800 FLEX (Radiometer). De-identified whole blood samples collected from clinical locations were analyzed for method comparison. Regression analysis was performed for each measured analyte according to Clinical Laboratory Standards Institute (CLSI) EP09-A3. Additionally, both systems were evaluated for operator usability in terms of system availability, troubleshooting, maintenance and service interventions. Observation and analyzer event logs were utilized for this analysis. **Results:** Regression results are summarized in table 1 below. For the analytes where a regression evaluation was not possible due to the limited sample range acquired during the study, the 95% confidence intervals of the bias results was calculated (Chloride, COHb and MetHb). Additionally, GEM Premier 5000 demonstrated excellent ease-of-use with laboratory personnel and provided a highly reliable service when measured in terms of analyzer availability compared to the ABL800. **Conclusion:** The GEM Premier 5000 system demonstrated good analytical performance compared to the ABL800. Methodology differences between analyzers contribute to subtle differences observed for some analytes but do not have any impact in clinical treatment. Strong analytical correlation, combined with system ease-of-use, through the all-in-one GEM PAK and iQM2, make the GEM Premier 5000 a suitable platform for the POC or a high throughput laboratory environment.

Analyte	N	Slope	Intercept	Regression Coefficient
pH	215	1.124	-0.907	0.955
pCO2	215	1.088	-0.941	0.978
pO2	214	1.031	4.116	0.995
Na+	215	0.944	7.509	0.935
K+	215	1.125	-0.35	0.996
Ca++	215	1.095	-0.099	0.978
Glu	214	1.07	-9.953	0.992
Lac	213	1.059	0.052	0.991
Total Hemoglobin	213	0.959	0.162	0.998
O2Hb	213	0.974	4.205	0.998
HHb	213	0.98	-1.638	0.998
sO2	213	0.974	4.139	0.998

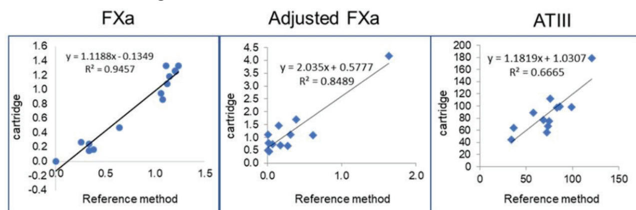
B-190

Digital Microfluidic Platform for Heparin Monitoring in Pediatric Patients using Small Volume Samples

S. Emani[1], C. Jacob[2], L. Nelson[2], A. Kennedy[2], R. Singh[2], S. Norton[2], R. Sista[2], V. Pamula[2], S. Emani[1]. [1]*Boston Children's Hospital, Boston, MA,* [2]*Baebies Inc., Morrisville, NC*

Background: Unfractionated heparin and heparinoids are frequently utilized for treatment or prevention of thrombosis in hospitalized children. Current laboratory methods for monitoring heparin levels include Factor Xa assay, which is used to adjust dosing accordingly. However, current assays require frequent, large volume blood draws that can lead to iatrogenic anemia and potentially further blood transfusions. In this study, we evaluate the feasibility of utilizing a prototype digital microfluidic system for near-patient monitoring of heparin in children. **Methods:** Functional assays for Antithrombin-III (ATIII), Factor Xa (FXa), and FXa function with exogenous ATIII supplementation (adjusted FXa) were adapted on the digital microfluidic platform by developing customized fluorogenic substrates and measuring fluorescence (360/460 nm). All assays were performed on the cartridge by mixing one droplet of diluted sample with a droplet of specific activator, incubated for approximately 2 min at 37°C, followed by addition of specific substrate and kinetic measurement. Discarded whole blood samples were obtained from hospitalized patients that were being treated with unfractionated heparin (N=12). All assays were performed on the microfluidic platform and the results were compared to those obtained using clinical reference method. Linear regression and Bland-Altman plots were utilized for comparisons. **Results:** FXa values obtained using the microfluidic method correlated well with those obtained using the clinical reference laboratory method (slope = 1.1188, R^2 = ~0.95) (Figure). The sample volume required is significantly lower for all on-cartridge tests compared to the clinical methods (25µL vs. 2mL) and so is the time to result (10 mins. vs. 2 hrs). **Conclusions:** This study demonstrates the feasibility of testing for heparin monitoring with high-fidelity using low sample volumes on a digital microfluidic platform. The technology offers the possibility of near-patient,

automated heparin monitoring in pediatric patients with minimal blood sample and rapid time to result. Application of this near-patient technology in clinical settings needs further investigation.



B-191

A Novel Newborn Screening Device for Illicit Drug Exposure

R. Sista, J. Coyne, A. Kennedy, R. Singh, S. Norton, V. Pamula. *Baebies Inc., Durham, NC*

Background: Neonatal abstinence syndrome (NAS) refers to a spectrum of withdrawal symptoms in newborns who were exposed to illicit substances *in utero*. Babies with NAS have higher rates of fetal anomalies and perinatal issues, which raise the risk for adverse long term outcomes. With approximately 5% of mothers using at least one addictive drug during pregnancy, rates of NAS continue to soar. To address this public health crisis, ACOG and AAP recommend universal screening of substance use in pregnancy using standardized behavioral scoring tools which are often biased due to subjective scoring. While toxicology testing for NAS is expanding, several factors restrict widespread newborn screening including: challenging methodologies for meconium and umbilical cord sample collection and processing and long turnaround times for results. The consequences of delayed or insufficient NAS screening include delays in pharmacologic treatments, high costs of unnecessary hospitalization, and increased risk for misdiagnosis. **Methods:** We propose a novel device and a workflow to enable rapid screening of urine or meconium samples in the hospital utilizing a disposable digital microfluidic cartridge on a small analyzer. Assays for five common classes of drugs (fentanyl, opioids, amphetamines, cocaine, and benzodiazepines) are performed on a fully automated cartridge. Droplets of liquids are programmably manipulated to perform enzyme modulated immunoassays (EMIT) where an analyte (one of the drugs) and G6PD-labelled-analyte compete for an antibody specific to the analyte. Binding of the analyte to the antibody displaces G6PD-labelled-analyte from the antibody and the free G6PD thereafter causes an increase in signal from turnover of NAD+ to NADH. The increase in kinetic fluorescence signal is proportional to the amount of analyte. **Results:** The EMIT assay is fully automated with a total time to result of less than 15 mins on the cartridge. The assay has a measurement range of 1 to 100 ng/mL, covering the necessary relevant range. **Conclusions:** A near-patient testing device is timely to address the rising incidence of NAS and we demonstrate feasibility of such a device for rapid NAS screening. The potential benefits include reduced length of hospitalization for unaffected newborns, accelerated time to confirmatory results, and faster resolution of acute withdrawal symptoms.

B-192

Rapid Diagnosis Platform Based on Combination of Microfluidic System and Homobifunctional Imidoester in Clinical Specimens

C. Jin, B. Koo, Y. Shin. *Asan Medical Center, Seoul, Korea, Republic of*

Background: In order to diagnose diseases accurately, the development of diagnosis techniques with high sensitivity and specificity is necessary. In the case of infectious disease, the concentration of pathogens in clinical samples (blood, saliva, urine, etc.) is low, which leads to difficulty in detection and diagnosis. In cancer diagnosis, the detection of point mutation is important criteria as decision for surgery or various treatment options. Despite recent advances in the rapid and accurate detection of pathogens or mutations, there is still a lack of technology that can be easily and cost-effectively applied. Here, we developed a rapid and accurate diagnostic platform based on homobifunctional imidoesters (HIs) as sample preparation technique which can be applied to various diseases.

Methods: HIs are nonchaotropic and crosslinker reagents which can be used to capture amine groups of nucleic acids (both DNA and RNA) as covalent bonds or to bind the surface of negatively-charged pathogens (bacteria and virus) as electrostatic coupling. HIs were combined with low-cost plastic thin film with microchannels for sample preparation. When a sample mixed with HIs is injected into the platform, it can enrich pathogens without additional instrumentation in 30 min. When the sample injected with HIs and lysis buffer, it can elute nucleic acids in less than 30 min. After the optimization of the technique, this system was tested with mammalian cells, bacterial cells, and various clinical samples (tissue, plasma, environmental swab, saliva and cerebrospinal fluid) for diagnosing infectious diseases and cancer.

Results: The new sample preparation technique using HIs significantly increased the pathogen enrichment and nucleic acid extraction rate. The technique does not require large instruments, and has improved amplification and time efficiency, portability, and affordability. We tested various samples infected with human parainfluenza, herpes zoster, tuberculosis; all the results showed high pathogen detection rates (2-4 times) compared with conventional column-based extraction methods. Moreover, we attempted to detect point mutation using liquid biopsy from cancer patients. The system captured cell-free DNA (cfDNA) including circulating tumor DNA with low background DNA. It also showed higher concordance in the status of point mutation between primary tumor and plasma samples by the HI platform (71.4%) compared with the column-based method (57.1%). Finally, we demonstrated the utility of the integrated system with sample preparation platform and isothermal bio-photonic sensor instead of PCR technology in various sample sources. This diagnostic system provides a rapid (<1.5 h) and sensitive (10-100 times higher than qPCR) detection sensor for genetic and epigenetic analysis with low cost and simplicity.

Conclusion: Our new diagnosis platform provides a simple, cost-effective, and ultra-sensitive pathogen diagnosis technique and capture nucleic acids simultaneously for use with both large and small volumes of clinical samples; thus, it has significant utility in clinical applications. Moreover, it can isolate cfDNA and detect point mutation in cancer samples with high sensitivity. Therefore, we believe that the rapid diagnostic system may change the paradigm of sample processing and nucleic acid detection systems in clinical applications.

B-193

Evaluation of Diagnostic Performance of Two Rapid Antigen Tests for Influenza

S. Lee, J. Kim, Y. Cho. *Yonsei University College of Medicine, Seoul, Korea, Republic of*

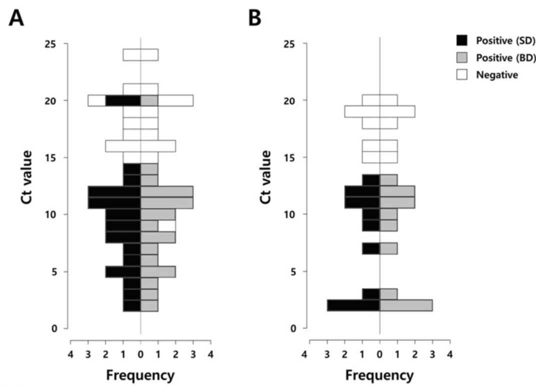
Background: Rapid influenza diagnostic test (RIDT) is widely used for the diagnosis of influenza owing to its simplicity and convenience of use. This study has been designed to evaluate the performance of two RIDT assays —BD Veritor Flu A + B (Becton Dickinson and Company Diagnostic, Sparks, MD, USA) and SD Standard F influenza A/B FIA (SD Biosensor, Inc., Suwon, Korea)— using the results of real-time reverse transcription PCR (rRT-PCR) as reference.

Methods: A total of 117 nasopharyngeal specimens obtained from patients with suspected influenza were tested for influenza infection by rRT-PCR and two RIDT assays. The results obtained from both RIDT assays were qualitatively compared with those from rRT-PCR which was used as the standard for reference.

Results: The Veritor™ and Standard F assays were found to demonstrate a sensitivity of 65.62% and 71.88%, respectively, for the detection of influenza A with a 100% detection specificity. Additionally, both assays demonstrated 66.67% sensitivity and 100% specificity for the detection of influenza B. A correlation was found between the cycle threshold (Ct) values from rRT-PCR analyses and the sensitivity of RIDT methods (Figure 1). Almost all samples with low Ct values, below 14.36 for influenza A and 13.26 for influenza B, were detected by both assays. One sample with Ct value of 9.79 is missed by BD Veritor assay. While seven influenza A positive samples with Ct values ranging from 15 to 20 were not detected, one with a Ct value of 20.07 was detected by both assays. One additional positive sample with a Ct value of 20.31 was detected only by the SD Standard F assay.

Conclusion: We are the first to evaluate a novel RIDT—the Standard F influenza assay which demonstrated a comparable sensitivity for the detection of influenza A and B with the BD Veritor assay.

Figure 1. Comparison of Ct values for influenza rRT-PCR-positive specimens (A: influenza A, n = 32; B: influenza B, n = 18). Specimens with negative rapid antigen test results are indicated by an empty box.



B-194

Clinical Performance of STANDARD F Strep A Ag FIA Test and Sofia Strep A FIA Test for Diagnosis of Acute Bacterial Pharyngitis

S. Kim[1], W. Choi[2]. [1]*Changwon Gyeongsang National University Hospital, Changwon, Korea, Republic of,* [2]*Department of Nursing Science, Kyungsung University, Busan, Korea, Republic of*

Background: Rapid and accurate diagnosis of bacterial pharyngitis is essential for optimal antibiotic treatment. Clinical performance of STANDARD F Strep A Ag FIA (SD Biosensor, Korea), a recently developed rapid antigen detection test (RADT), and Sofia Strep A FIA Test (Quidel, USA), a currently widely used RADT worldwide, was evaluated for patients with pharyngitis.

Methods: One-hundred forty five patients with sore throat visiting five pediatric clinics in Changwon, Korea were subjected to throat swabs three times during 2018-2019. Two swabs were used for both RADTs and the other swab was delivered to Gyeongsang National University Changwon Hospital (GNUCH) for bacterial culture. Bacterial culture was regarded as a standard test. This study was approved by IRB of GNUCH and all participants agreed on written consent.

Results: Sensitivity, specificity, positive predictive value (PPV), and negative predictive value of STANDARD F Strep A Ag FIA were 95.2%, 91.1%, 64.5%, and 99.1%, respectively and those of Sofia Strep A FIA Test were 100%, 93.6%, 72.4%, and 100%, respectively compared to bacterial culture. Positive agreement and negative agreement revealed 84.6% and 99.1% respectively.

Conclusions: STANDARD F Strep A Ag FIA exhibited an excellent clinical performance except PPV and relatively a good agreement with Sofia Strep A FIA Test. Discrepant result with culture might be due to sampling order, bacterial numbers of group A , and delayed transport. This is an ongoing clinical study and more collection of data will improve the PPV and positive agreement.

B-195

A Novel, Point-of-care (POC), Hand-Held Meter & Strip, Viscoelastic Coagulation Diagnostic Platform for Thromboelastography (TEG) and Anticoagulation Therapy Monitoring

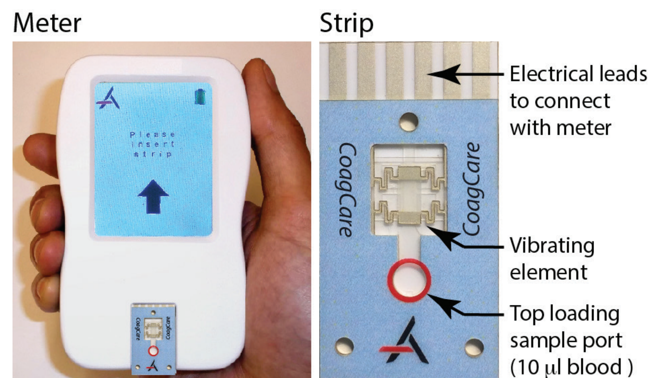
A. Ramkumar, N. Yoshimizu, E. Brennan, J. Merselis. *Abram Scientific, Inc., Menlo Park, CA*

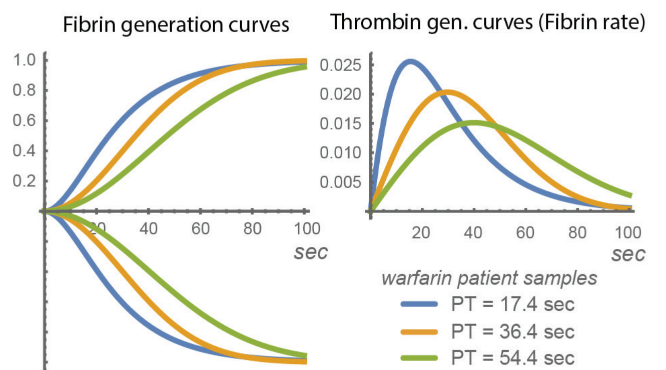
Background: The CoagCare, a viscoelastic hemostatic diagnostic platform, comprised of a hand-held POC meter and test strips, is presented here. The strip incorporates a highly-sensitive element vibrating (1-10μm) at high frequency (1-5KHz), and measures the viscoelastic properties in a 10μl blood sample, thus enabling the entire TEG curve and parameters to be evaluated in under 5 minutes. Further, conventional coagulation diagnostic tests, such as prothrombin time or international normalized ratio (PT/INR), activated partial thromboplastin time (aPTT), and activated clotting time (ACT), are also enabled in addition to a contemporaneous density measurement to measure hematocrit.

Methods: Citrated venous whole blood from normal subjects spiked with varying amounts of heparin (0-5IU/ml), rivaroxaban (0-500ng/ml), dabigatran (0-500ng/ml), eptifibatide (0,4μg/ml), and tissue plasminogen activator (tPA,0-1.8nM) were tested on the CoagCare system using extrinsic (tissue factor) and intrinsic (kaolin) test strips, and the TEG curve/parameters, aPTT and ACT tests were recorded. Further, the PT/INR was evaluated using finger-stick whole blood from normal subjects and warfarin patients compared to the Roche CoaguChek® system.

Results: The following parameters were extracted from a typical CoagCare TEG curve: Clot Formation Time (TEG-R), Activated Clotting Time (TEG-ACT), Peak Thrombin Generation (ThrombinPeak), Clot Stiffness (G, [fibrinogen]^2), Platelet Contraction (TEG-Plt-Cont), and Fibrinolysis Rate (TEG-Lys-Rate). TEG-ACT seconds increased proportionally and Peak Thrombin Generation decreased exponentially with increasing heparin up to 3IU/ml. While Clot Stiffness remained relatively constant and Peak Thrombin Generation decreased with increasing rivaroxaban concentration, dabigatran showed a pattern of decreasing Clot Stiffness and relatively constant Peak Thrombin Generation. Further, PT/INR exhibited good correlation with Roche's CoaguChek® over 1-3INR range. Linear response was observed to heparin concentrations for aPTT (0-1IU/ml) and ACT (0-3IU/ml,1-5IU/ml).

Conclusion: CoagCare successfully demonstrated the capability to generate a TEG curve and parameters in under 5 minutes, in addition to conventional coagulation tests, thus enabling rapid, bedside diagnostics for fast and efficient clinical management.





B-196

An Unexpected Laboratory Confirmed Mixed, Acute Alkalosis in a Emergency Department Patient with a Strong History of Diabetic Ketoacidosis

S. Asadbeigi, K. E. Blick. *Un of OK Health Sci Ctr, Oklahoma City, OK*

Background: Diabetic ketoacidosis (DKA) is typically the first concerning acid-base disorder among patients with diabetes. These patients also can present acutely with other less common mixed and complicated acid-base disorders. Since these mixed

acid-base disorders need proper diagnosis and treatment, accurate laboratory data and proper interpretation in a timely manner is a crucial factor for diagnosis. We present a case of mixed acid-base disturbance with severe alkalosis in a long-standing insulin dependent diabetic resulting in a combination of respiratory and metabolic alkalosis. Since the patient had a history of DKA, this unexpected severe alkalosis made a challenging case for the clinical team leading them to question the accuracy of our point-of-care (POC) laboratory results. **History:** The patient is a 53 year-old Hispanic female with history of insulin-dependent diabetes and end-stage renal disease. She presented to the emergency department with abdominal pain, nausea and vomiting, and shortness of breath. The initial arteria gas analysis showed pH of 7.65; PCO_2, 21.4 mmHg; and $HCO3^-$ of 23 mEq/L. The POC testing on the Abbott i-Stat were as follows: glucose, 700 mg/dL; lactic acid, 3.40 mmol/L; sodium, 129 mEq/L; and a decreased potassium level of 2.9 mEq/L. Ionized calcium and chloride were also significantly decreased. The anion gap was normal even though the lactate was slightly elevated.

Results: In the presented patient, metabolic and respiratory alkalosis with mild metabolic lactic acidosis caused an acute alkalotic state which is atypical for a patient presenting in our ED with a very high glucose level. In this diabetic patients, DKA was the first clinical impression, even though this patient had high pH, normal anion gap, and a normal plasma beta hydroxybutyrate and a negative urine ketone test making DKA unlikely. The clinical team remained doubtful about the POC i-Stat lab results and accordingly ordered confirmation with Core lab testing which showed the same severe alkalosis. Indeed, the patient's week-long shortness of breath with hyperventilation and productive cough was consistent with the observed respiratory alkalosis along with dehydration and electrolyte imbalance due to recent vomiting causing a concomitant metabolic alkalosis: the latter made persistent due to electrolyte imbalance. With fluids and treatment for the hypokalemia and hypochloridemia, the alkalosis improved significantly in spite of the fact that the patient had suboptimal renal function. **Conclusion:** An initial clinical diagnosis of acute DKA in our diabetic patient with severe alkalosis significantly delayed treatment when physicians mistakenly distrusted the accuracy of our POC laboratory testing. The treatment of this diabetic patient with an acutely high presenting glucose was effective only after lowering the blood glucose level and also balancing fluids and electrolytes in order to permit the renal system to more effectively compensate her alkalosis.

B-197

An Evaluation of the i-Stat B-Type Natriuretic Peptide (BNP) for the Rapid Point-of-Care Assessment of Congestive Heart Failure and Acute Coronary Syndromes (ACS) in the Emergency Department

S. Asadbeigi, A. Esmaeili, K. E. Blick. *Un of OK Health Sci Ctr, Oklahoma City, OK*

Background: Blood levels of B-type natriuretic peptide (BNP) are used as one of the major markers of congestive heart failure and acute coronary syndromes (ACS). The plasma BNP levels are also elevated in patients with acute ischemic stroke. Rapid and accurate diagnostic Point-of-Care (POC) BNP testing for early-state identification of cardiovascular abnormalities has become an essential tool for bedside testing in many Emergency Departments (ED). Indeed, an increase in the concentration of key cardiovascular biomarkers like BNP in circulating blood immediately after a myocardial event like CHF and/or ACS complements other essential tools including clinical history, signs and symptoms, electrocardiograms, chest X-rays, and other different and less-critical Core laboratory-based assays. Done as part of our project of implementing faster bedside BNP testing to improve patient cardiovascular triage in our ED, we present a comparison study of the Abbott i-Stat BNP versus our Core laboratory BNP testing on the Siemens Centaur XP.

Methods: We evaluated the accuracy of the I-Stat quantitative BNP assay with a comparison study on 21 patient samples (some of which were spiked to get higher levels) using the BNP method on our Core laboratory Centaur XP as a reference. Whole blood was tested on the i-Stat and heparinized plasma was used for the Centaur BNP. Precision studies included replicate analysis (n=15 per sample pool) on specimens from known healthy donors and spiked to low, mid and high BNP levels.

Results: While the i-Stat BNP method showed a high level of correlation when compared to the Centaur XP reference method (I-Stat = 1.54*Centaur XP + 2.80; n=21, $r^2 = 0.991$, Syx = 31.2 ng/mL), the observed regression line slope of 1.54 shows the results for the i-Stat BNP were 1.54 times higher than the Centaur XP therefore indicating that results from these two methods are not interchangeable. Accordingly, reference ranges for the i-Stat BNP had a higher 154 ng/mL cutoff reflecting the slope of 1.54 observed and the essentially zero Y-intercept. The i-Stat BNP precision was acceptable and consistent throughout the assay range with CVs of 5.39% (at 79.4 ng/mL, n=30), 5.76% (999 ng/mL, n=30), and 5.47% (2,552 ng/mL, n=30).

Conclusion: We conclude that 1) the rapid BNP on the i-Stat with results in 10 minutes at the bedside shows excellent correlation with our Core laboratory Centaur XP method and 2) the i-Stat BNP has an acceptable level of accuracy and precision. However, diagnostic cutoffs for CHF must be adjusted to reflect the significant proportional error observed between these two methods. We have also noted that that after implementation of the i-Stat BNP, the ED length-of-stay for triage of CHF patients was significantly improved. For ACS patients, BNP is also measured along with troponin-I on the first sample of our timed (Zero-2 hour) and rapid ED chest pain “rule-out” protocol.

B-198

Comparison of Capillary versus Venous Blood on ALERE AFINION AS100 Analyzer for Hemoglobin A1c

A. Alanazi, C. Bueno, N. Alhussain, F. Santos, N. Nicolas, A. Alharbi, W. Tamimi. *Department of Pathology & Laboratory Medicine, King Abdulaziz Medical City, Riyadh, Saudi Arabia*

Background: Control of diabetic patients requires measuring of the hemoglobin A1c (Hgb A1c) in their blood which will precisely estimates the average glucose level during the period of three months for those patients and their compliance. The gold standard for measuring Hgb A1c includes High Performance Liquid Chromatography (HPLC) and capillary electrophoresis. However, these techniques require venous blood samples and longer time to be reported back to health care provider. There is a need to provide the results in shorter turnaround time (TAT)with less invasive blood collection techniques. The point of care testing (POCT) analyzers may assist health care providers to improve the management of diabetic patients. In this study we have evaluated ALERE AFINION AS100 analyzer and compared it to Variant100 HPLC for Hgb A1c in venous and capillary blood samples. **Methods:** Venous and capillary samples were collected from 30 diabetic patients simultaneously. The capillary samples were immediately processed in the ALERE AFINION AS100 analyzer using bronate affinity and the result were reported. The venous samples collected in the EDTA tubes were split into two parts. The first part were performed immediately on ALERE AFINION AS100 analyzer and the second part was transported to the main lab and processed by HPLC (Variant100, BioRad) on the same day. All statistical analysis were done on both EP Evaluator and Microsoft Excel sheet and the cutoff of significance for p value was selected to be less than 0.01. The total allowable error for accepting the results was selected to be +/- 6 % According to NGSP and CAP guidelines. The cutoff value for any abnormal results was selected at 6.5% for Hgb A1c **Results:** The comparison of venous samples performed on both ALERE AFINION AS100 and Variant 100 analyzers has showed an excellent correlation (r=0.9944) however it was significantly difference (p<0.00001). The mean average were found to be 8.00±2.3 and 7.60±1.19 respectively. However, the Hgb A1c on ALERE AFINION AS100 have shown higher values than those of Variant100, BioRad by 5.6%. Moreover, the bias difference increased to 7.33% above the cutoff value at the abnormal decision and decreased to 2.64% at the normal decision cutoff value. Only two values were found to be discordant by using this cutoff value. The comparison of capillary and venous samples has also showed an excellent correlation (r=0.9940) between ALERE AFINION AS100 and Variant 100 analyzers respectively (p=0.015). The mean average were found to be 8.17±2.46 and 7.99±2.31 respectively. Again though lower, the bias for Hgb A1c on ALERE AFINION AS100 have shown higher values than those of Variant100, BioRad by 3.9% but no significant differences was observed above or below the cutoff value. All values between capillary and venous were concordant by using this cutoff value. **Conclusion:** The POCT Hgb A1c ALERE AFINION AS100 analyzer using capillary samples is comparable to the gold standard HPLC and can provide accurate results in easy and shorter TAT.

B-199

Rapid and Accurate Multiple Detection Bio-Optical Sensor for Diagnosis of Emerging Infectious Diseases

B. Koo, C. E. Jin, Y. Shin. *Asan Medical Center/Asan Medical Institute of Convergence Science and Technology, Soeul, Korea, Republic of*

Background: Emerging infectious diseases (EIDs) such as *Coxiella burnetii*, scrub typhus, severe fever with thrombocytopenia syndrome (SFTS), MERS-CoV, Zika and Ebola have increased due to changes in various environmental and ecological factors and are a major problem in the world public health. Rapid and accurate diagnosis of EIDs is important in the prevention of further infections and in the treatment of EIDs patients. In addition, multiple detection techniques that simultaneously detect two or more targets in a single reaction are essential for the diagnosis of EIDs by reducing

time and reagent costs. However, existing multiplex diagnostic methods have several limitations such as low sensitivity, high cost, time-consuming and high technicality. Here, we developed an isothermal, label-free, one-step nucleic acids amplification and multiple detection bio-optical sensor for the diagnosis of EIDs.

Methods: We have prepared a recombinase polymerase amplification (RPA) solution capable of nucleic acid amplification under isothermal (37-42 °C) conditions to amplify and detect target nucleic acids. The target sequence was hybridized with the forward DNA probe immobilized on the silicon mircoring resonator (SMR) surface, and the target was amplified by the RPA reagent on thermoelectric cooler (TEC). The wavelength is shifted over time by the amplified target product, and measured in label-free real-time manner. Various clinical samples (nasopharyngeal, tissue and blood plasma) were used to optimize bio-optical sensor for the diagnosis of EIDs.

Results: This bio-optical sensor shows rapid (<20min) and high sensitivity without any labeling and in a real-time manner for detection of EIDs using clinical samples. We compared the detection limits of bio-optical sensors using several samples (*C. burnetii*, scrub typhus and SFTS) and found that they were 10-100 times more sensitive (10-100 copies/reaction) than conventional PCR methods (10^3 copies/reaction). Furthermore, we have demonstrated high sensitivity (>90%) in clinical samples (liver biopsy and blood plasma) of *C. burnetii* infected patients. This bio-optical sensor is capable of multiplex detection by immobilizing DNA probes that recognize different target sequences in the three individual microrings acting as sensor. We have verified that multiple detection of bio-optical sensor using MERS-CoV, Zika and Ebola samples is possible and that the detection sensitivity (10-100 copies/reaction) is 10-100 times higher than the conventional PCR method (10^3 copies/reaction). Moreover, the bio-optical sensor clearly distinguished positive and negative in 20 clinical nasopharyngeal samples including 11 MERS-CoV and Human coronavirus OC43 infected patients.

Conclusion: We have developed a new isothermal nucleic acids solid-phase amplification and multiple detection assay system for rapid (<20min), simple, and label-free detection of EIDs. This bio-optical sensor is highly sensitive and specific and has proven to be applicable to a wide variety of EIDs. Moreover, the clinical utility of the assay to identify multiple pathogens in clinical specimens is useful to overcome the limitation of conventional approaches.

B-200

Evaluation of the Performance of Five Automated Urinalysis

A. G. Marques[1], F. C. G. Nunes[2], P. C. M. Koga[2], C. E. S. Ferreira[2], A. M. Doi[2], M. D. V. Martino[2], J. Pasternak[2]. [1]*Hospital Albert Einstein, sao bernardo do campo, Brazil,* [2]*Hospital Albert Einstein, sao paulo, Brazil*

Background: Urine test is one of the oldest laboratory tests. Composed by dipsticks and urinary sediment, the automated analysis guarantees process standardization, substantial reduction of the analyst subjectivity beyond provides quick results to the patient. The aim of this study was compare the performance of five automated urine equipments.

Methods: We conducted a research and found seven suppliers with equipment that performs automated urine tests. Five of these, offered their equipment for this study of performance and all reagents required were be provided. The equipment were: LabUMat 2™ and UriSed 3™ (77 Eletronika - Hungary) represented by Abbott, Attelica 1500™ (Siemens Healthineers - Germany), Dirui FS 200™ (Dirui Company - China) represented by Scenika Disgnosticos and Sysmex Series UM™ - (Sysmex - Japan). As a gold standard, we used the equipment validated and currently in use in our laboratory, iRICELL 3000™ (Iris Diagnostics, a Beckman Coulter company - USA). Were compared the following parameters for dipstik: 1) Specific gravity (SG), 2) pH, 3) Protein, 4) Glucose, 5) Ketones, 6) Bilirubin, 7) Urobilinogen, 8) Nitrite, 9) Leucocyte Esterase and 10) Hemoglobin. For sediment, were compared leukocytes (WBC) and erythrocytes (RBC) counts per microliter of urine. The other parameters such as casts, bacteria, yeasts and crystals were not evaluated since in none of the technologies are identified automatically by the equipments. P values less than 5% was considered statistically significant. **Results:** Were calculated Kappa index (KI) for dipsticks (n=585). In all of them, the KI was greater than 0,8 demonstrating an excellent correlation as follows: Protein (kI=0,863), Glucose (kI=0,812), Ketones (kI=0,923), Bilirubin (kI=0,909), Urobilinogen (kI=0978), Nitrite (kI=0,818), Leucocyte Esterase (kI=0,803) and Hemoglobin (kI=0,834). Specific gravity, pH, WBC and RBC was performed ANOVA analysis: SG (p=0,07) and pH (p=0,00048). Although statistically significant, in rare cases the pH measured between the equipment was clinically different. In the vast majority of cases, the differences were between acid pH (5.0, 5.5 and 6.0) and basic pH (7.0, 7.5 and 8.0) measurements. For the sediment (n=488), there was no significant statistical difference to WBC (p=0,33) and RBC (p=0,45). **Conclusion:** Despite different technologies, all the equipment tested presented satisfactory comparative results. The choice of platform can therefore be based on criteria such as price, processes and availability of equipment by the supplier.

B-201

National Investigation of the Imprecision of POCT Glucose Meter

L. Jin, W. Wang, F. He, K. Y. Du, S. Yuan, Z. Wang. *National Center for Clinical Laboratories, National Center of Gerontology, Beijing Hospital, Beijing, China and Graduate School of Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, Beijing, China*

Background: POCT glucose meter is widely applied to diabetic patients, which necessitate investigation and analysis of imprecision of blood glucose meter. However, opinions varied on the reliability of POCT glucose meter. This study aims to help to get an comprehensive understanding of imprecision of POCT glucose meter in Chinese laboratories.

Method: The laboratories' CV and SD during 2015 to 2018 were collected from the EQA software developed by the NCCL of China. Based on ISO 15197:2013, the criteria of TEa for POCT glucose meter was presented in separate glucose concentration intervals: for glucose concentrations <5.55 mmol/l, TEa was defined as SD≤0.83mmol/l; for glucose concentrations ≥5.55mmol/l, TEa was defined as CV<7.5%. Accordingly, 1/4 TEa and 1/3TEa were calculated. Then, percentages of laboratories meeting quality requirement were calculated according to 1/3TEa, 1/4TEa.

Results: After eliminating invalid data, there were 189, 620, 687, 857 laboratories submitted their data from 2015 to 2018, respectively. Results were presented in the table. As we can see, percentages of laboratories participating external assessment increased from 2015 to 2018. Majority of participant laboratories obtained good CV. Percentages of laboratories whose current CV met 1/3TEa from 2015 to 2018 were separate, when glucose concentrations <5.55mmol/l, they were 87.18%, 83.40%, 84.19%, 86.58%, respectively; when glucose concentrations ≥5.55mmol/l they were 77.48%, 81.27%, 75.12%, 82.29%, respectively. Percentages of laboratories whose CV met 1/4TEa at glucose concentrations <5.55mmol/l from 2015 to 2018 were 51.28%, 67.22%, 78.26%, and 79.53%, whereas percentages were 54.95%, 49.08%, 52.30%, and 58.86% when glucose concentrations ≥5.55mmol/l was employed, respectively. However, percentages of laboratories meeting imprecision requirement were all below 90%, which might be a room for improving.

Conclusion: More and more laboratories realized the necessity of EQA for POCT. But it's still a long journal to travel for advancing in the management of POCT.

Table The Imprecision of POCT Glucose Meter During 2015 to 2018

Year	Number of participant laboratories	Current CVs of Chinese laboratories			Allowable Imprecision specifications based on ISO 1597:2013			
					1/3TEa		1/4TEa	
		Median	25th	75th	glucose concentration <5.5mmol/l	glucose concentration ≥5.5mmol/l	glucose concentration <5.5mmol/l	glucose concentration ≥5.5mmol/l
					SD≤0.28	CV<5%	SD≤0.21	CV<3.75%
2015	189	4.12	2.59	5.80	87.18% (68/78)	77.48% (86/111)	51.28% (40/78)	54.95% (61/111)
2016	620	3.77	2.50	5.10	83.40% (201/241)	81.27% (308/379)	67.22% (162/241)	49.08% (186/379)
2017	687	4.08	2.50	5.42	84.19% (213/253)	75.12% (326/434)	78.26% (198/253)	52,30% (227/434)
2018	857	3.50	2.40	4.90	86.58% (258/298)	82.29% (460/559)	79.53% (237/298)	58.86% (329/559)

B-202

Point-of-Care Analytic Performance versus Central Laboratory

M. L. De Angelis, R. Buonocore, A. R. Corno, E. Inglese, L. Mondrone, S. Nerini Molteni, A. Longhi, W. P. Porreca, C. F. Perno, S. Pastori. *ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy*

Background: The College of American Pathologists (CAP) defines Point-of-Care Testing (POCT) as "testing that is performed near or at the site of a patient with the result leading to a possible change in the care of the patient". Guidelines require that POCT devices provide the most reliable results without significant differences from those obtained with the central laboratory analytical instruments. We compared the analytical performances of a blood gas versus laboratory auto-analyzers.

Methods: Sodium, chloride, potassium, bicarbonates, glucose and lactate concentrations were determined on 97 heparinized venous blood samples with one RAPIDPoint® 500 Systems (Siemens Healthcare, Germany) and, simultaneously, with a Cobas®8000 analyzer (Roche, Germany); hemoglobin was measured on 94 samples with RAPIDPoint 500 and on one Sysmex XN1000 (Dasit, Italy). All samples (61 male/36 female) were collected in a 3-month period from hospitalized subjetcs and outpatients from different settings.

Analytical accuracy was evaluated by Passing-Bablok regression and Bland-Altman difference analyses (MetComp rev1.0 by M. Vidali, SIBIOC).

Results:

analyte	Passing-Bablok regression equation	95%CI for slope and intercept, respectively	Bland-Altman bias	95%CI for bias
Sodium mmol/L	Y=14.180+0.880X	0.727/1.040; -8.320/35.773	3.26	2.56/3.95
Chloride mmol/L	Y=21.960+0.800X	0.672/0.935; 8.168/35.059	-1.36	-2.04/-0.68
Potassium mmol/L	Y=-0.004+0.992X	0.942/1.042; -0.204/.0.204	0.02	-0.021/0.06
Bicarbonates mmol/L	Y=1.017+1.083X	0.951/1.242; -2.330/3.912	-3.28	-3.74/-2.81
Glucose mg/dL	Y=0+1.000X	0.980/1.032; -3.452/2.020	0.01	-0.86/0.88
Lactate mmol/L	Y=0.052+0.785X	0.715/0.865; -0.138/0.211	0.45	0.39/0.50
Hemoglobin g/dL	Y=1.888+0.882X	0.769/0.967; 0.968/3.177	-0.057	-0.51/0.40

Passing-Bablok: presence of a systematic difference and of a proportional difference for chloride and hemoglobin; presence of a proportional difference for lactate; for all the other analytes regression provided no differences between methods.

Bland-Altman: Statistically differences were observed for sodium, chloride, bicarbonates, and lactate. All differences met the Proficiency Testing (CLIA, 1992) criteria: ±4 mmol/L for sodium; ± 0.5 mmol/L for potassium; ±5% for chloride; ±6 mg/dL (or ±10%) for glucose, ±7% for hemoglobin (no cut-off value available for bicarbonates and lactate); however, 60.6% paired samples for hemoglobin were above the CLIA cut-off value.

Conclusion: The data obtained, to be integrated by precision evaluation, showed analytical agreement between the POCT RAPIDPoint 500 and the Laboratory analyzers for all tested parameters; however, interchangeability of the POCT device with the central laboratory analyzers need further evaluation from a clinical perspective.

B-203

D-dimer, Presepsin and qSOFA for Early Assessment of Organ Dysfunction and Mortality Prediction in Patients Admitted with Sepsis to the Emergency Department

E. Spanuth[1], P. Engesser[2], B. Ivandic[3]. *[1]DIAneering Diagnostic Engineering & Research, Heidelberg, Germany, [2]Mitsubishi Chemical Europe, Düsseldorf, Germany, [3]Department of Medicine III, University of Heidelberg, Heidelberg, Germany*

Background: Coagulation activation during sepsis can initiate multiple organ failure. D-dimer as a highly sensitive marker of coagulation activation may early indicate the presence of sepsis induced organ failure. Presepsin has been shown to provide early prognostication in sepsis. The qSOFA score defined by the Third International Consensus Definitions for Sepsis and Septic Shock can be assessed at patient admission without laboratory tests. The qSOFA score has been shown to be associated with an increased probability of mortality and can be used for prognostication. Aim of the study was to compare qSOFA and the POC tests D-dimer and presepsin (PSEP) at admission for assessment of organ dysfunction and differentiation of sepsis, severe sepsis or septic shock or risk of mortality.

Methods: 99 Patients admitted with signs of sepsis were included. Sepsis induced organ dysfunction, severe sepsis or septic shock were defined according to current guidelines. The qSOFA score was calculated from respiratory rate, GCS score and systolic blood pressure using the recommended thresholds: respiratory rate ≥ 22/min, altered mentation (GCS<15), systolic blood pressure ≤ 100 mmHg. The DIC score was established according to ISTH (International Society on Thrombosis and Hemostasis) guidelines. D-dimer, PSEP and NT-proBNP were determined using the PATHFAST™ platform from LSI Medience Corporation. Creatinine, CRP and lactate were measured by using laboratory methods.

Results: Discrimination between patients with uncomplicated sepsis (n=73, mortality=2.7%) and the high risk group with severe sepsis or septic shock (n=50, mortality=34%) revealed AUC values of 0.592, 0.620, 0.639, 0.682, 0,688, 0.762 and **0.830, 0.853** for CRP, lactate, D-dimer, NT-proBNP, PSEP, qSOFA and the simultaneous assessment of **qSOFA+D-dimer** or **qSOFA+D-dimer+PSEP**, respectively. 19 patients died during hospitalization. AUC values of mortality prediction were 0.517, 0.605, 0.665, 0.708, 0748, 0.842 and **0.848, 0.876** for CRP, D-dimer, NT-proBNP, lactate, PSEP, qSOFA, and the simultaneous assessment of **qSOFA+D-dimer** and **qSOFA+D-dimer+PSEP**, respectively. Comparison of the DIC score and D-dimer revealed higher discriminatory validity of D-dimer for prediction of death and severe sepsis or septic shock. The AUC-values for DIC score and D-dimer for prediction of death were 0.514 vs. 0.605 and for discrimination between uncomplicated sepsis and severe sepsis or septic shock 0.526 vs. 0.639, respectively. Using the threshold ≥2 of qSOFA, ≥500 ng/L of D-dimer and > 500 ng/L of PSEP, qSOFA detected 18 non-survivors (95%) and 41 patients of the high-risk group (82%), D-dimer detected 17 non-survivors (89%) and 33 (66%) patients of the high-risk group (n=50) and PSEP detected 18 non-survivors (95%) and 45 patients of the high-risk group (89%).

Conclusion: The simultaneous assessment of qSOFA+D-dimer or qSOFA+D-dimer+PSEP provided added value to assess the severity and early mortality risk in patients admitted with sepsis to the emergency department. Combining qSOFA and D-dimer or PSEP improved the validity significantly. These parameters could be determined already at admission without time delay as D-dimer and PSEP could be measured as POC assays in parallel in anticoagulated whole blood samples using the PATHFAST™ system within 16 min.

B-204

Clinical Utility of Point-of-Care Tests for Determination of Urinary Albumin Excretion

C. R. Garlipp, P. V. Bottini, D. G. S. Foga. *University of Campinas, Campinas, SP, Brazil*

Background: Microalbuminuria is the excretion of small quantities of albumin insufficient to be detected by the methods usually employed to measure urinary protein. In both insulin-dependent and non-insulin-dependent diabetes, microalbuminuria predicts the onset of proteinuria and chronic renal failure. Although microalbuminuria may be an early feature of diabetic glomerular disease, it also occurs in non-diabetic subjects, especially in the presence of essential hypertension and associated with vascular disease. Thus, the accurate measurement of urinary albumin excretion (UAE) is of great clinical importance.

Several quantitative methods such as nephelometry, immunoturbidimetry, enzyme linked immunosorbent assay (ELISA) and chemiluminescence (CLIA) are available. Recently point-of-care tests (POCT) for the evaluation of urinary albumin excretion immunoassay have been developed. One of them is a semi-quantitative reflectance screening test (CLINITEK® Microalbumin 2 reagent strip) that measures the concentrations of albumin and creatinine whose results are expressed as albumin / creatine ratio. Results between 30 to 300 mg/g should be confirmed by quantitative methods for albumin. Another point-of-care test is the DCA® Microalbumin/Creatinine urine test which is a quantitative assay that provides results in a few minutes.

Methods: This study aimed to evaluate the CLINITEK® Microalbumin 2 reagent strips and the microalbumin/creatinine DCA® assay as semi-quantitative and quantitative confirmatory assays respectively, comparing the results obtained with the traditional methods for UAE analysis (nephelometry for albumin / BN Prospect and colorimetric modified Jaffé method for creatinine / AU5800). Samples with less than 30 mg/dL of protein (Multistix® 10 SG) were submitted to the CLINITEK® Microalbumin 2 screening test. Samples whose screening test was positive (albumin / creatinine ratio between 30 and 300 mg/g) were submitted to the confirmatory assay (DCA® Microalbumin / Creatinine urine test).

Results: For comparison purposes, the diagnosis of microalbuminuria was based on the results obtained by the quantitative nephelometric and colorimetric assays. Statistical analysis revealed a strong correlation between CLINITEK® Microalbumin 2 reagent strip and the traditional quantitative assays (r=0.8696, kappa=0.6980, p<0.0001) discriminating microalbuminuria and normal UAE (<30mg/g) with sensi tivity=82%,specificity=89%, predictive positive value=77% and negative predictive value=92%. DCA® Microalbumin/Creatinine urine test showed excellent correlation with AUE results obtained by nephelometry / modified Jaffé methods (r=0.9951)

Conclusion: In summary, our data demonstrate that the combined use of semi-quantitative and quantitative point-of-care tests provides a useful alternative for ruling out microalbuminuria, being rapid, accurate and easy to perform.

B-205

Evaluation of Whole Blood Basic Metabolic Panel Assay with Pediatric Sample

C. Xu[1], D. Cross[1], M. White[2], A. Bollinger[2], D. Dietzen[2]. *[1]Instrumentation Laboratory, Bedford, MA, [2]Washington University in St. Louis, and St. Louis Children's Hospital, St. Louis, MO*

Background: A Basic Metabolic Panel (BMP) is one of the most commonly ordered blood tests that provides physicians with a quick assessment of a patient's electrolyte and fluid balance, blood glucose concentration and kidney function. A whole blood (WB) BMP cartridge based on electrochemical creatinine, blood urea nitrogen (BUN), and total CO_2 (tCO_2) assays is being added to the blood gases, electrolytes and metabolites test menus currently offered on the GEM Premier analyzers (Instrumentation Laboratory/IL). The goal of this clinical evaluation is to compare the WB analytical performance of the GEM Premier BMP cartridge for pediatric samples in a POC setting to the established reference methods.

Methods: Remnant pediatric WB samples were obtained from the cardiovascular operating room (CVOR) at St. Louis Children's Hospital and evaluated by POC staff. The WB samples were analyzed on the GEM Premier analyzer (IL) with five BMP cartridges over the course of three months. As reference methods, the WB samples were then assayed on a GEM Premier 4000 analyzer (IL) for Na^+, K^+, iCa^{++}, Cl^-, glucose, lactate, pH, pCO_2 and hematocrit. The plasma portions were assayed on the Cobas 6000 analyzer (Roche Diagnostics) for creatinine (enzymatic assay), BUN, and tCO_2. Some of the native WB samples were adjusted to expand the measured ranges.

Results: The WB results from GEM Premier BMP cartridges correlated well with those obtained from reference analyzers across the tested ranges; the results from regression analysis and the biases estimated from regression equation at each Medical Decision Level (MDL) per analyte are summarized in Table 1.

Conclusion: Strong correlations were observed between the GEM Premier BMP and the reference methods for all the analytes evaluated from pediatric samples. GEM Premier BMP cartridge can provide reliable results using minimal blood volumes with quick turnaround time in POC environments, such as the CVOR.

Table 1. Method Correlation Results for the GEM Premier BMP WB Assays *vs.* References [(a)] **Roche Cobas 6000 or** [(b)] **GEM Premier 4000 by** [(c)] **Passing-Bablok or** [(d)] **Deming Regression Analysis**

Analyte	Slope	Intercept	r	N	Sample Range	MDL1 (Bias)	MDL2 (Bias)	MDL3 (Bias)
BUN [(a,c)]	0.951	0.177	0.997	155	3.0 - 102.9 mg/dL	6.0 (-0.12)	26.0 (-4.3%)	50.0 (-4.6%)
Crea [(a,c)]	1.000	-0.025	0.997	146	0.20 - 13.07 mg/dL	0.6 (-0.025)	1.6 (-0.025)	6.0 (-0.4%)
tCO_2 [(a,c)]	1.000	0.250	0.986	155	5.8 - 44.8 mmol/L	6.0 (0.25)	20.0 (1.3%)	33.0 (0.8%)
Na^+ [(b,d)]	1.014	-1.149	0.990	147	100 - 167 mmol/L	115 (0.4)	135 (0.7)	150 (0.9)
K^+ [(b,c)]	1.000	0.100	0.999	149	0.5 - 14.8 mmol/L	3.0 (0.10)	5.8 (0.10)	7.5 (1.3%)
Cl^- [(b,c)]	1.000	1.000	0.995	148	45 - 139 mmol/L	90 (1.1%)	112 (0.9%)	n/a
iCa^{++} [(b,c)]	0.964	0.053	0.999	150	0.36 - 3.76 mmol/L	0.37 (0.04)	0.82 (0.024)	1.58 (-0.2%)
Hct [(b,d)]	1.041	-1.074	0.996	146	16 - 69 %	21 (-0.2)	33 (0.3)	55 (1.2)
Glu [(b,c)]	1.020	-0.836	0.999	147	46 - 684 mg/dL	45 (0.1)	90 (1.3%)	180 (1.5%)
Lac [(b,c)]	1.000	-0.200	0.998	147	0.6 - 16.0 mmol/L	2.0 (-0.2)	5.0 (-4.0%)	n/a
pH [(b,d)]	1.011	-0.076	0.993	185	7.03 - 7.87	7.30 (0.002)	7.35 (0.002)	7.45 (0.003)
pCO_2 [(b,c)]	1.000	0.000	0.995	188	7 - 119 mmHg	35 (0.0)	50 (0.0)	70 (0.0%)

B-206

Incidence Rates of Critical Low Glucoses (<40 mg/dL) by POCT before and after New Policies for Treatment of Clinically Significant Hypoglycemia (>54 mg/dL): A Comparison between Two Hospitals

L. M. Evans, D. F. Stickle, B. M. Goldsmith. *Thomas Jefferson University Hospital, Philadelphia, PA*

Background: Glucose <54 mg/dL was recently defined as Clinically Significant Hypoglycemia (CSH, American Diabetes Association, *Standards of Medical Care in Diabetes*, 2017). In January 2018, our hospitals instituted a call-back policy for inpatient CSH from the central laboratory, and instituted a new nursing procedure for response to CSH. We examined whether the new policies had affected the incidence rate of low glucose critical values (CRITICAL, <40 mg/dL) among POCT glucose measurements. This was investigated for two hospitals within our system: A, a 950-bed academic medical center hospital, and B, a 200-bed community hospital.

Methods: POCT glucose results (Roche Accuchek) for two 12 week intervals before (PRE) and after (POST) the policy change date were obtained from the POCT reporting system for both hospitals. Incidence quotients (Q, defined as results either in category of CRITICAL or CSH per total number of glucose results in interval) were tabulated by Excel spreadsheet for PRE and POST dataset analyses.

Results: Analyses were performed for four datasets (table below). The most important statistic is a comparison of the number of unique patients experiencing one or more CRITICALS in intervals PRE and POST. Q among unique patients having one or more CRITICALS was significantly increased in POST for hospital A.

Conclusion: After adoption of new CSH policies, the incidence quotient (Q) for CRITICAL glucoses by POCT increased significantly at hospitals A and B by 26% and 28%, respectively ($p<0.01$). Q for unique patients having one or more CRITICALS increased at hospital A by 17% ($p<0.01$) and at hospital B by 7% (not significant). Presumably, the increases reflect increased awareness of circumstances in which CSH may develop, leading to a significantly higher selection rate for measurement of CRITICAL glucoses by POCT. POCT for glucose clearly plays an important role in response to CSH at both hospitals.

Hospital A 950-bed academic medical center hospital	**PRE**	**POST**	**Comments: POST compared to PRE**
Total	n=76,745 (6,450 patients)	n=78,838 (6,507 patients)	
Critical	n=169 (97 patients) Q (Criticals): 2.20E-3	n=218 (116 patients) Q (Criticals): 2.77E-3	Q increased by 26%
CSH	n=613 (318 patients) Q (CSH): 7.99E-3	n=769 (328 patients) Q (CSH): 9.75E-3	Q increased by 22%
Unique patients (one or more critical results)	n=97 Q (Unique): 1.26E-3	n=116 Q (Unique): 1.47E-3	Q increased by 17%
Hospital B 200-bed community hospital	**PRE**	**POST**	**Comments: POST compared to PRE**
Total	n=16,954 (2,434 patients)	n=19,779 (2,589 patients)	
Critical	n=26 (16 patients) Q (Criticals): 1.53E-3	n=39 (20 patients) Q (Criticals): 1.97E-3	Q increased by 28%
CSH	n=101 (59 patients) Q (CSH): 5.95E-3	n=110 (54 patients) Q (CSH): 5.56E-3	Q decreased by 7%
Unique patients (one or more critical results)	n=16 Q (Unique): 0.94E-3	n=20 Q (Unique): 1.01E-3	Q increased by 7%

B-207

Evaluation of Urine Ovulation Kits for Point of Care Testing

W. S. Setoh, C. K. M. Ho, R. X. L. Yang, C. Loh, C. A. Khoo, V. Veronique. *KK Women's and Children's Hospital, Singapore, Singapore*

Background: Ovulation is the release of an egg from the ovary into the fallopian tube where it is ready to be fertilized. Detecting ovulation in advance is important for women seeking pregnancy. Prior to ovulation, the body produces a large amount of luteinizing hormone (LH) which triggers the release of a ripened egg from the ovary. Urine ovulation kits are commonly used to detect the presence of LH in urine. In this study, we aimed to evaluate the performance of five different urine ovulation kits i.e. Abon, Fortel, Ascen and Artron 113 and Artron 130 with the following: 1) Concor-

dance with Clearblue kit 2) Concordance with serum LH result. 3) Ease of reading the result4) Ease of use **Methods:** A total of fifty female patient urine and blood samples were collected. The urine samples were tested with the different urine ovulation kits by the nurses, admin staff and doctors. A scoring matrix was used to assess the ease of use and ease of reading of the test kit results. The blood samples were tested for serum LH in the laboratory using Abbott Architect i2000SR analyser. **Results:** The results from both Artron 113 and 130 kits were 96% concordance with Clearblue. The Clearblue concordance results for Fortel, Abon and Ascen kits were 88%, 84% and 66% respectively. The results from both Artron 113 and 130 were 94% concordance with serum LH tests. Serum LH concordance results for Fortel, Abon and Ascen kits were 86%, 82% and 68% respectively. Artron 113 kit was the easiest to read with a rating of 84% while Abon kit was the easiest to use with a rating of 100% from the scoring assessments. **Conclusion:**

All the five different urine ovulation kits were evaluated. Artron 113 kit was shown to have the best overall performance.

B-208

Point-of-Care Microvolume Cytometer Measures Platelet Counts with High Accuracy from Capillary Blood

E. Y. Chan[1], W. M. Dickerson[1], P. Schatz[2], H. Westergren[2], J. Paraskos[3], A. Tigerstrom[2], R. Yu[1], J. MacMunn[1], J. Cheng[1], L. Li[1]. *[1]rHEALTH, Cambridge, MA, [2]AstraZeneca, Gothenburg, Sweden, [3]AstraZeneca, Cambridge, United Kingdom*

Background: Point-of-care testing (PoCT) of platelet count enables physicians to have real-time data that facilitates rapid decision making and has great potential to improve outcomes in patients at risk for thrombocytopenia. **Methods:** Fingerprick and venous blood samples from healthy volunteers (N = 31) were analyzed using a new USB-powered miniature cytometry-based blood analyzer, the rHEALTH ONE device, which requires only 8 µL sample to provide absolute volumetric platelet counts. Data were compared with the International Society of Laboratory Hematology (ISLH) platelet method, which requires a cytometer and a clinical impedance analyzer. Interfering substances and conditions were tested and compared, including RBC fragments, platelet fragments, cholesterol, triglycerides, lipids, anti-platelet antibodies, and environmental temperature. **Results:** A concordance between the rHEALTH ONE and ISLH methods with a slope = 1.030 and R^2 = 0.9684 was observed. In addition, the rHEALTH ONE method showed a correlation between capillary and venous blood samples (slope = 0.9514 and R^2 = 0.9684). Certain interferents changed platelet recovery: RBC fragments and anti-platelet antibodies with the ISLH method; platelet fragments and anti-platelet antibodies on the rHEALTH ONE; and RBC fragments, platelets fragments, triglycerides and LDL on the clinical impedance analyzer. The precision of the rHEALTH ONE method ranged from 3.1 - 8.0% for physiological platelet counts. **Conclusions:** The rHEALTH ONE method provides similar results compared to established ISLH method as well as good correlation between capillary and venous blood samples. This demonstrates the ability of the rHEALTH ONE to provide point-of-care assessment of platelet count from fingerprick blood with high precision and limited interferences.

B-209

Development of an Automated Multiplex Assay for the Rapid Identification of Non-Tuberculosis *Mycobacterium* from Clinical Samples

M. S. Conover, H. Liu, L. Wagner, K. L. Heflin, J. C. Wells. *iCubate Inc., Huntsville, AL*

Background: *Mycobacterium tuberculosis* is an extremely prevalent pathogen with over 1 billion individuals being infected worldwide. However, in the United States, the disease burden is much lower with only about 10,000 *M. tuberculosis* cases reported annually. Interestingly, cases of Non-Tuberculosis *Mycobacterium* (NTM) generally equal or exceed the *M. tuberculosis* cases throughout much of the developed world. NTM generally infects immuno-compromised individuals, but are increasingly being found in otherwise healthy patients. Diagnosis of these infections can be very slow and complicated. Culture can take up to 6 weeks to grow some NTM species and subsequent biochemical speciation can often be unclear. These factors, combined with the extensive antibiotic treatment and possible quarantine for individuals with suspected *Mycobacterium*, highlights the need for faster and more accurate diagnostics.

Methods: iCubate has developed a novel multiplex PCR technology, ARM-PCR, that allows for the specific detection of pathogens from complex samples. ARM-PCR chemistry has been incorporated into a closed platform, the iC-Cassette, and automated on the iC-System. This platform has allowed for the development of an NTM assay that identifies 6 of the most common NTM species that cause pulmonary infections.

Results: Preliminary inclusivity tests were performed against 72 clinical isolates, 12 for each target, and demonstrated 98.6% agreement with the clinical result. Sensitivity of the assay was tested by diluting each species in decontaminated sputum with the goal of detection below 1x10^5 cfu/ml to accommodate the lower limit of acid-fast stain detection. Finally, exclusivity was performed by testing a panel of more than 60 non-target *Mycobacterium*, normal flora and pathogens commonly present in sputum samples.

Conclusions: The results of these tests indicate that the iCubate NTM assay displays sufficient specificity, sensitivity, and inclusivity to accommodate the general needs of clinical labs performing NTM diagnostics. This multiplex assay has the potential to be a breakthrough in NTM diagnostics by saving weeks of diagnostic time and thereby improving the treatment and quality of life for patients worldwide.

B-210

Evaluation of Measured Creatinine and eGFR by a Point-of-Care Whole Blood Enzymatic Assay

C. Xu[1], Z. Bellio[1], M. Curley[1], C. McLeod[2], F. Kirkland[2], W. Clarke[2], P. Stack[3], M. Souriyayong[3], S. Abdirizak[3], K. Schulz[3], S. Love[4]. *[1]Instrumentation Laboratory, Bedford, MA, [2]Pathology, Johns Hopkins Medical Institutions, Baltimore, MD, [3]Hennepin Healthcare, Minneapolis, MN, [4]Hennepin Healthcare, and University of Minnesota, Minneapolis, MN*

Background: Estimated Glomerular Filtration Rate (eGFR) from serum creatinine is considered a better assessment of renal function than serum creatinine alone. Patients with chronic kidney disease have a higher risk of developing contrast-induced nephropathy (CIN). A quick creatinine/eGFR screening test is beneficial in patient risk management. A whole blood (WB) IDMS traceable electrochemical enzymatic creatinine assay, along with eGFR based on the Modification of Diet in Renal Disease (MDRD) Study equation and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, is an addition to the blood gas, electrolyte and metabolite test menus currently offered on the GEM Premier analyzers (Instrumentation Laboratory/IL). The goal of this clinical evaluation is to compare the analytical performance of the GEM WB creatinine assay and the clinical concordance of eGFR in a point-of-care (POC) setting to the established reference method.

Methods: Remnant heparinized WB samples along with age and sex information were obtained from Coronary Care Unit, Emergency Department, Intensive Care Unit, Oncology, Operating Room and other areas at Johns Hopkins Medical Institutions (MD, USA) and Hennepin Healthcare (MN, USA). The WB samples were analyzed by POC staff on the GEM Premier analyzer (IL) with creatinine assay on five cartridges over the course of two months. The plasma portions were assayed on Cobas Integra analyzers (Roche Diagnostics) with IDMS traceable enzymatic creatinine assay as the reference method. The eGFR was estimated for both WB and plasma creatinine by applying the IDMS traceable versions of the MDRD and the CKD-EPI equations. Since ethnicity (African American/AA or non-African American/non-AA) was unknown for individual samples, all four possible eGFR values (MDRD AA, MDRD non-AA, CKD-EPI AA, CKD-EPI non-AA) were calculated for the purpose of evaluating the clinical concordance, for both Cobas plasma and GEM WB creatinine for each patient sample. The clinical concordance of categorizing patients with abnormal kidney function (WB eGFR<60 ml/min/1.73 m^2) and overall concordance of patients (eGFR<60+eGFR≥60) vs. plasma eGFR were assessed.

Results: A total of 287 patient samples were analyzed, with 154 males and 133 females. The age range of patients was from 18-89 years with a mean of 50 years (±SD 19 years). The GEM WB creatinine correlated well with plasma samples on the Cobas across the tested ranges with the Passing-Bablok regression result of: GEM_WB_Creatinine = 1.019*Cobas_Creatinine - 0.036, r=0.996. A mean bias of -0.014 mg/dL between GEM and Cobas was obtained from Bland-Altman analysis. When identifying patients with abnormal kidney function (eGFR<60), GEM WB creatinine showed 97% (87/90) and 81% (61/75) concordance with plasma creatinine by applying MDRD non-AA and AA equations, respectively. Conversely the CKD-EPI non-AA and AA equations showed concordance of 91% (80/88) and 84% (62/74), respectively. The overall clinical concordance of 98% (280/287) and 94% (271/287) with MDRD non-AA and AA equation, 97% (277/287) and 95% (272/287) with CKD-EPI non-AA and AA equation were observed, respectively.

Conclusion: Strong correlations were observed between the GEM Premier WB creatinine and eGFR versus the reference method. GEM Premier WB creatinine assay provides reliable results with quick turnaround times in POC setting for rapid renal function assessment.

B-211

Multi-Mediator Reagent Formulation for Use in Electrochemical Detection

D. Deng, G. Neel. *Omni Biomedical, Weston, FL*

Background: Blood glucose assays are a valuable tool to manage diabetes. For patients being monitored using finger stick or continuous glucose sensors with a single electron mediator based enzyme assay, changing result values due to high background current stability, low assay sensitivity and oxygen dependence can have significant implications for therapy. To overcome these issues of single mediator assays, a novel approach using the synergistic effect from a combination of mediators (osmium and ruthenium) was discovered to overcome the shortcomings of each individual mediator to achieve a desirable combination of properties and assay performance. **Methods**: The reagent formulation for detecting glucose: 50 mM phosphate buffer; 10,000 U/mL glucose oxidase (GOx): 150 mM ruthenium hexamine trichloride; 5 mM [Os(III)(bpy)$_2$imCl]Cl$_2$); 0.25% w/v hydroxypropyl methylcellulose; and 0.06% w/v Triton X-100. The reagent formulation was micro-deposited onto screen printed carbon sensors and dried at 50°C for 4 minutes. The sensors were then tested for oxygen dependence using venous whole blood samples, which were divided into two portions. One portion was kept as is and sealed airtight (pO_2 at 37 mmHg), while the other portion was oxygenated to a desired pO_2 level (e.g., 120 mmHg, 215 mmHg, or 226 mmHg). The pO_2 values for all blood samples were measured using a blood gas analyzer (OPTI Medical Systems, Inc.). Blood samples at each pO_2 level were further divided into 5 aliquots and spiked with glucose to target a range of glucose concentrations from 100 to 550 mg/dL. Actual glucose levels for each aliquot were assayed using an YSI glucose analyzer (Yellow Springs Instruments). The blood samples were then applied to the sensors by capillary fill (n=10), and the glucose dose response was measured using a potentiostat with an excitation voltage of 0.40V. **Results:** Surprisingly, it has been found that a mediator formulation comprising both a ruthenium and an osmium mediator (e.g., Ru(NH$_3$)$_6$Cl$_3$ and [Os(III)(bpy)$_2$imCl]Cl$_2$) with GOx produced consistent dose responses with minimal O_2 dependence for pO_2 values ranging from 37 to 215 mmHg. **Conclusions:** The novel multi-mediator formulation can be successfully used with glucose oxidase (GOx) with surprisingly low oxygen interference and minimal measurement bias, providing an improvement over each mediator used alone. The synergistic effects of this multi-mediator (Super Mediator) formulation are applicable to a wide range of analyte/enzyme pairings, and are not limited those described.

B-213

Development of POCT System of Measuring the Blood Zinc Concentration

T. Sugiyama, R. Takeuchi, J. Ando, T. Ohta, S. Watabe, T. Ohtake. *TAUNS laboratories, Inc., Izunokuni-shi, Shizuoka, Japan*

Background: Zinc is an essential element for maintaining life and is an important constituent of many enzymes. The deficiency of zinc in vivo causes symptoms such as taste disorder, male sexual dysfunction, and developmental disorder. Treatment of zinc deficiency is carried out by administration of zinc preparation, but it is required to measure zinc concentration in the blood before prescribing of zinc preparation in Japan. (Zinc Deficiency Guideline 2018 of the Japanese Society of Clinical Nutrition) Measurement of zinc concentration in the blood is performed by atomic absorption spectrometry or colorimetric method, but it has not been possible to measure the zinc concentration at the medical treatment site and immediately reflect the result in medication. Therefore, we report on development of POCT system. The system comprises an assay for measuring the fluorescence of a zinc-porphyrin complex using a porphyrin compound as a detector reagent for blood zinc ions and a portable fluorescence measurement apparatus for measuring said fluorescence.

Methods: It was prepared standard solution to prepare a zinc sulfate solution 80 µg / dL. We prepared the zinc solution at 2 to 8 µg / dL, and the above range is the range of zinc concentration in the sample at the measurement. We selected 5,10,15,20-Tetrakis(N-methylpyridinium-4-yl)-21H,23H-porphine, tetrakis(p-toluenesulfonate) (hereinafter referred to as TMPyP.) as a detector reagent, which was added to the prepared zinc solution and reacted at room temperature, the fluorescence generated from the zinc-TMPyP complex to excited by 430 nm wavelength was measured at 621 nm. Also, to a serum specimen of known zinc concentration, a predetermined concentration of zinc sulfate solution was added to sample, and prepared quasi specimens of various zinc concentrations. A sample treatment solution containing a surfactant and a reaction promoter was added to above samples, and prepared a 20-fold diluted sample. Subsequently, TMPyP was added and fluorescence was measured.

Results: We were able to measure the zinc ion concentration in the buffer in the range of 2 to 8 µg / dL in the above POCT system. We confirmed that the measured value has linearity in the above range. The results show that can measure the range of 40 to 160 µg/dL as the blood concentration of zinc. We measured the fluorescence of a zinc-TMPyP complex at quasi serum sample and confirmed that it is the same result as the control experiment. In addition, it was a result suggesting the possibility of obtaining the same result in the specimens collected from the patients.

Conclusion: We developed an assay that can measure the concentration of zinc in serum samples in the range of 40 to 160 µg / dL and have semi-quantitative properties. We believe that the system consisting of this assay and the portable fluorescence measurement device can provide serum zinc concentration information conveniently and quickly at the clinical site and can contribute to the decision on whether to administer the zinc preparation. We also believe that this POCT system can be applied to other metal ion detection.

B-214

Preanalytical/Biologic /Analytical Variation of Patients Tested with the iSTAT Compared to Patients Tested with Gem 4000, Radiometer and Siemens for Electrolytes, Glucose and Blood Gases

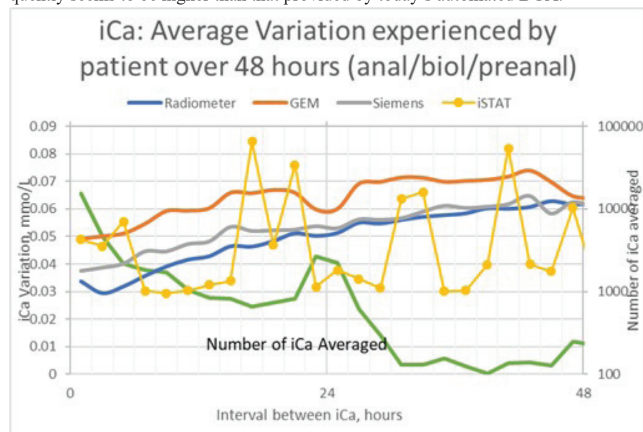
J. Mei[1], G. Cembrowski[2], M. Cervinski[3], H. Sadrzadeh[4], R. Schifman[5]. *[1]University of Manitoba, Winnipeg, MB, Canada, [2]University of Alberta, CC Quality Control Consulting, Emonton, AB, Canada, [3]Giesel School of Medicine, Hanover, NH, [4]Calgary Laboratory Services, Calgary, AB, Canada, [5]University of Arizona, Veterans Administration Hospital, Tucson, AZ*

Introduction: For many of today's electrolyte/metabolite/blood gas analyzers (BGA), the user has little comprehension of their analytical goodness, especially when they are operated in acute care environments. We have developed a methodology that transforms sequential intrapatient test results into a measure of total PreAnalytic (including biologic) and ANalytic variation (PAAN) (ClinBiochem 2017;50:936-941). We have used this methodology to retrospectively demonstrate analyzer superiority or inferiority over the short term and more recently extended this examination period to days, weeks and months for tandem IL Gem 4000s, tandem Radiometer ABL800s and a single Siemens RapidPoint500. In this presentation, we present PAAN graphs for 9 analytes measured by the iSTAT analyzer with data extracted from 15 US Veterans Hospitals gathered between 12/2016 and 12/2018. These iSTAT PAAN graphs are compared to those of the other 3 POC analyzers.

Methods and Materials: For each analyte (Na, K, Cl, HCO3, iCa. Glucose, ph, PO2 and PCO2) and each analyzer, we tabulated all possible pairs of intra-patient truncated results separated by time intervals of 2-4, 4-6, . . . up to 48 hours. The average variations in these 2-hour groups of paired components were calculated using the standard deviation of duplicates (SDD) formula. The resulting SDD values were graphed against the midpoints of the respective two-hour interval.

Results: The Figure compares iSTAT iCa 48 hour PAAN variations to the PAAN of the other 3 BGA. The variation of iSTAT tests that are repeated every one or two hours almost always exceeds or equals that of the inferior BGA analyte. When tests are done less frequently, there is lesser variation. Beyond 24 hours there is more variation which is an artifact of the lesser numbers of test differences.

Discussion: iSTAT variation for critically patients where results are repeated frequently seems to be higher than that provided by today's automated BGA.



B-215

The Clinical Usefulness of ABL90 FLEX PLUS Blood Gas Analyzer for Rapid Measurement of Creatinine and Urea According to Sample Type

S. Lee, H. Choi, S. Kee, S. Kim, M. Shin. *Chonnam National University, Gwangju, Korea, Republic of*

Background: Creatinine (Cr) and urea testing at the point of care could help healthcare providers rapidly assess the renal status of a patient. ABL90 FLEX PLUS blood gas analyzer (Radiometer, Denmark) is designed to offer Cr and urea test results along with blood gas testing. The purpose of this study is to compare Cr and urea results measured by ABL90 FLEX PLUS with the results of automatic chemistry analyzers in clinical laboratories using various blood samples used in clinical practice.

Methods: A total of 105 patients were recruited from Chonnam National University Hwasun Hospital in Korea and blood was obtained simultaneously in heparin tube, sodium-citrate tube, and serum-separating tube. The Cr and urea results of heparinized whole blood (WB), sodium-citrate treated WB, and serum measured by ABL90 FLEX PLUS, were compared with those of four chemistry analyzers: Cobas 8000-c702 (Roche Diagnostics, Switzerland), Hitachi 7600-210 automatic analyzer (Hitachi, Japan), AU5822 chemistry analyzers (Beckman Coulter, USA), ADVIA 1800 chemistry system (Siemens Healthcare GmbH, Germany). The results were analyzed by one-way analysis of variance, linear regression analysis, and Bland-Altman method comparison.

Results: The mean value of Cr and urea showed no statistically significant difference by sample types including heparinized WB, sodium-citrate WB and serum, using ABL90 FLEX PLUS (mean ± SD: 1.15 ± 1.08, 1.01 ± 0.95, and 1.19 ± 1.11 in Cr, 22.72 ± 17.11; 18.71 ± 13.58; 22.13 ± 20.29 in urea). In linear regression analysis using the results between ABL90 FLEX PLUS and each automatic chemistry analyzer, the slope of sodium-citrate WB (mean, 0.82 of Cr and 0.69 of urea) was lower than that of heparinized WB (mean, 0.94 of Cr and 0.87 of urea) and serum (mean, 0.96 of Cr and 1.03 of urea) samples ($P<0.5$). The coefficient of determination (R^2) was above 0.99 for Cr and ranged from 0.94 and 0.98 for urea. In Bland-Altman method comparison with each of 4 chemistry analyzers, the average value of mean ± SD of Cr (and urea) bias (%) was -5.00 ± 12.49 (and -9.82 ± 9.32) in heparinized WB, -17.76 ± 9.79 (and -28.40 ± 10.24) in sodium-citrate WB, and -0.76 ± 10.31 (and -18.97 ± 12.13) in serum. The mean bias of Cr and urea results in heparinized WB or serum were smaller than sodium-citrate WB ($P<0.5$).

Conclusion: The Cr and urea of heparinized WB (as a recommended specimen) tested by ABL90 FLEX PLUS showed good agreement with each result of four automatic chemistry analyzers used in routine clinical practice. For measurement of Cr and urea by ABL90 FLEX PLUS, heparinized WB or serum specimen is more suitable than sodium-citrate WB. Therefore, a serum specimen, which is an easily accessible sample in clinical chemistry laboratories, can be used for rapid evaluation of renal function in ABL90 FLEX PLUS blood gas analyzer.

B-216

Exploratory and Multivariable Data Analysis Techniques for Evaluation of QC Data. A Practical Example for pH QC Performed with i-STAT G3+®, CG4+®, and CG8+ ® Cartridges

V. M. Genta[1], M. Kiger[1], S. F. Shumate[2], Y. Shen[1], L. Wyer[2]. [1]*Sentara Virginia Beach General Hospital, Virginia Beach, VA,* [2]*Sentara Healthcare, Norfolk, VA*

Background: In our Hospital, CG4+®. CG8+® and G3+® i-STAT cartridges are used interchangeably to evaluate blood pH, pCO_2, and pO_2 at the bedside. The QC is performed monthly by a nurse, transferred electronically to a microprocessor and analyzed with Minitab® statistical software using univariate and multivariate statistical techniques. **Materials:** Instruments: i-STAT cartridges CG4+, CG8+, G3+ (Abbott), several lot numbers. Quality Control Material: Level 1 Lot #22101712, Exp. Date 3/31/2019; Level 2 Lot #2210371, Exp. Date 3/31/2019 (i-STAT, Abbott). The QC assays were performed monthly by nurses performing POCT assays. The QC data were electronically transferred to AlereRALS® System (Alere) and then electronically transferred to Minitab® (Version 17, Minitab, Inc.) statistical software for exploratory and multivariable data analysis with graphic representations. **Results:** While the parallel box plot for pH level 2 showed homogeneity of mean and variability for 8 months, for pH level 1 the box for CG8+ for the month of October showed both a shift to higher values and an increase in variability. The data for CG8+ were extracted from the data base. This visual impression was corroborated by quasi-normal distribution with skewness toward higher values, ANOVA, Tukey's multiple comparisons of means, and tests for equality of variances with Bonferroni's 95% confidence intervals (CI). Both the Shewart's mean and EWMA QC charts detected this shift. The standard deviation chart by month clearly demonstrated a statistically significant increased variance for the month of October (Multiple Comparisons P<0.0001, Levene's P<0.0001). The scatterplot by operator and date identified the operator associated with the increased variability and mean shift. Interestingly, the parallel box plots for pCO_2 Level 1 clearly showed a shift toward lower values and a statistically significant increase in variance (Multiple Comparisons P<0.0001, Levene's P<0.0001) for the same date and operator. This indicated that the QC vial was left uncapped causing evaporation of CO_2 and an increase in pH. The POCT Senior Technologist then promptly coached the operator. Repeat QC assays performed with the same QC control lot and cartridge lot numbers under supervision of the POCT Senior Technologist showed that the problem was corrected. **Conclusions:** Exploratory data analysis with parallel boxplots, allowed the immediate detection of a cluster of data exhibiting both a mean shift and increased variance among QC data performed using CG4+, CG8+, and G3+ i-STAT cartridges. This was confirmed by inferential statistical techniques. The electronic database allowed for the immediate extraction of data to answer the questions who, when, how much, and where. Additionally it allowed us to easily compare the pattern of one measurand (pH) with that of an inversely correlated measurand (pCO_2). In this practical example, the performance of a nurse at one POCT site in our hospital could be easily identified in real time and remedial action could be properly instituted. The availability of electronic software for acquisition and transmission of actionable QC and demographic data coupled with Minitab statistical software for data analysis was of paramount importance for answering who, what, when, where, how much, in an actionable period of time.

B-218

Comparison of Different Methods for the Determination of Sodium and Potassium Concentration in Chronic Kidney Diseases

K. R. *INSTITUTE OF NEPHROUROLOGY, BANGALORE, India*

Background: Acid-base and electrolyte alterations are common in patients with Chronic kidney disease (CKD).As the kidneys play a central role in the regulation of body fluids, electrolytes, and acid-base balance, CKD predictably results in multiple derangements including hyponatremia, hyperkalemia, metabolic acidosis and other metabolic derangements which, in turn, are intricately linked to morbidity and mortality. For a renal patient, electrolytes and arterial blood gas analysis are crucial and are monitored more frequently. Hence, accuracy in their results is at paramount importance. Variation of results for sodium and potassium can lead to incorrect patient evaluation or treatment. The most widely used method for electrolytes measurement is ion selective electrode (ISE), by direct potentiometry (direct ISE) or indirect potentiometry (indirect ISE). A number of studies have been done to compare the two methods especially in critically ill patients but none from an exclusive renal center, hence the objective of the present study was to investigate whether the results from ABG analyzer and autoanalyzer (AAs) are comparable when used to assess electrolyte levels (sodium and potassium) from patients with CKD especially in routine day-to-day scenario.Materials and methods: This was a retrospective study in which data were collected from simultaneous ABG and venous electrolytes measurements at the clinical laboratory of the Department of Biochemistry, Institute of NephroUrology, Bangalore during January 2019. A total of 242 samples were included in the study, where the venous and arterial electrolytes was measured by indirect ISE in Abbott ci4100 autoanalyzer and blood gas platform by direct ISE, Cobas b121 from Roche Diagnostics respectively. Results: The mean sodium level measured by AA was 132.62±5.70 mmol/L compared to 129.68±7.89 mmol/L in ABG. Pearson's correlation coefficient was 0.548 (p<0.0001). The Bland-Altman 95% limits of agreement for Na were -10.1 to 16.0 were very wide and clinically unacceptable. The mean potassium level measured by AA was 4.45±1.12 mmol/L compared to 4.22±1.02 mmol/L in ABG with associated Pearson's correlation coefficient of 0.849 (p<0.0001). The Bland-Altman 95% limits of agreement for K were -0.8 to 1.3 and clinically acceptable. Conclusion: Our study findings are in agreement with other studies which have shown that sodium results from AA and ABG are not equivalent and cannot be used interchangeably, whereas potassium results were in good agreement. But it does not overrule the importance of a simultaneous follow-up sample especially in severe and critical disorders where efficient electrolyte status determination, is vital.

B-219

An Investigation to Assess the Sensitivity Limits for Pregnancy Testing Kits and Devices - An 8 Year Study

S. Jones, G. J. Davies, N. Thomas, N. Blount, M. A. Thomas. *Cardiff and Vale UHB, Cardiff, United Kingdom*

Introduction Pregnancy testing kits or devices are used routinely in the UK both by health care professionals in clinical settings and by patients for home use. Studies have shown that the detection limits vary greatly between kits and the stated manufacturer's claims regarding their sensitivity are not always justified. A sensitivity study was carried out by Weqas in 2010, and was repeated in 2018.

Aim To assess the sensitivity limits for Pregnancy Testing kits and devices registered on the Weqas Urine Pregnancy Testing EQA Programme (Proficiency testing). To assess performance improvement and % uptake on reader technology over an 8 year period.

Method For both studies, urine was collected from healthy, non pregnant female volunteers, filtered to 0.2µm and Gentamycin added to maintain sterility. Intact hCG was added to the urine to provide pools across a concentration range of 0 - 50 IU/L. The pools were sent out to over 500 participants in 2010 and over 1300 in 2018.

Results 2010: At an hCG concentration of 30 IU/L, i.e. above the claimed positive cut off for all methods, 3 methods reported < 90% positivity. A further 4 methods, reported positive rates of 90 to 95% at this concentration. No method achieved 100% positivity rate at this concentration. **2018:** At an hCG concentration of 30 IU/L, 4 methods reported < 90% positivity. A further 5 methods, reported positive rates of 90 to 95% at this concentration whilst 3 others reported positivity rates of >96%. The 2 machine read devices reported 100% positive results.

Conclusions For the 2018 study the BioSign hCG DXpress and Clinitek Status device users consistently reported 100% positive results for all pools with hCG concentration above the stated cut-off whilst no visually read method achieved this. Approximately 25% of sites were using a strip reader device in 2010, compared to 50% in 2018. For the visually read kits (for both studies), although all the methods achieved > 50% positive rates at the claimed cut off, the consistency of the positive rates varied greatly between the methods. There was, however, a marked improvement in positivity rates at all concentrations in the 2018 study.

B-220

Wrangling the Wild West: Standardizing Ambulatory Point of Care Testing in a Large Healthcare System

A. Woodworth, D. Paskovics, P. Lee-Miller, B. Miller, K. Brown. *University of Kentucky Healthcare, Lexington, KY*

Background: Point of Care Testing (POCT) in ambulatory clinics is a quick, simple, non-invasive, affordable laboratory testing option that streamlines management of patients. Ambulatory POCT is regulated by the Clinical Laboratory Improvement Act (CLIA), the Office of Inspector General (OIG), and Centers for Medicaid & Medicare Services (CMS), and in some clinics, The Joint Commission (TJC) to ensure quality results. Clinics must document compliance with these agencies' regulations. Harmonization of POCT platforms, supplies and regulatory compliance streamlines patient care and reduces costs; however, standardization of POCT across an affiliated hospital system is challenging because of significant variability in clinic leadership, practices, patient populations, and resources. In this study, we describe the enterprise wide initiative at the University of Kentucky (UK) Medical Center to standardize POCT across ambulatory clinics. Led by a transdisciplinary team, this multifaceted project saw the successful standardization of regulatory compliance, testing platforms, and supply distribution.

Methods: The multidisciplinary team comprised of laboratory, ambulatory and operational specialists first completed a systematic review to document regulatory compliance, POCT methods/platforms and supplies. An evidence based review was conducted to choose the best methodology for each POC assay. New methods were validated according to laboratory policy prior to distribution. Clinic rounding with direct observation, engagement and intentional teaching (implicit and explicit) were utilized to acquire/maintain proof of certification and facilitate implementation of new platforms, quality policies, and training and competencies for testing professionals. After standardization, the team participated in ongoing monitoring of ambulatory clinics via quality control audits and site visits. The team also negotiated with supply chain and vendors to ensure uniform reagent ordering, distribution, and quality checks to increase cost efficiency and improve testing inventory availability.

Results: The initial needs assessment found 88 (43 stand alone and 45 travel) UKHC clinics performing 21 different POC tests. Eight tests on 7 platforms were standardized with 279 testing personnel trained and compliant on these methods. All clinics have obtained or migrated to appropriate CLIA certification. Efforts to standardize reagent distribution and supply chain have led to a uniform supply vendor.

Conclusions: This two year standardization effort has somewhat tamed the Wild West. Eight POCT assays have been standardized. The relationships built between the laboratory and clinical teams has expedited conflict resolution and increased patient care efficiencies. Monthly monitoring of clinics via quality control audits or site visits by POCT laboratory experts has improved compliance and patient safety. Standardized supplies have improved pricing and the pledge toward enhanced quality oversight. The ambulatory POC team has systematically worked to "tame the Wild West" through standardization. These efforts have resulted in improved patient care, a reduction in waste, streamlined supply distribution, and cost savings for ambulatory clinics in the UK Healthcare Enterprise.

B-221

Electrolyte and Metabolite Testing on GEM4000 Using Plasma and Serum Samples

J. Tang, G. Watts, Y. Chen. *Dr. Everett Chalmers Regional Hospital, Fredericton, NB, Canada*

Background: Core laboratory chemical analyzers are generally considered the gold standard in terms of electrolyte and metabolite testing. However, due to budget stringency, many hospitals - especially in more rural areas - have had to resort to cabbing samples off to nearby regional centers for their instrument downtime, an expensive process itself with long turnaround times. Using blood gas (BG) analyzers as backups for core laboratory analyzers has the potential to greatly reduce turnaround times and costs for blood tests assessing electrolyte and metabolite concentrations. We sought to evaluate the analytical performance of plasma and serum electrolyte and metabolite testing on GEM4000.

Methods: One plasma separator tube (PST) and one serum separator tube (SST) were drawn from 42 healthy individuals. Blood electrolytes and metabolites were analyzed with GEM4000 and Roche Modular P chemistry analyzer and compared for paired measurements, and their differences were assessed for Wilcoxon signed-rank test and for clinical significance.

Results: For Na^+, K^+, Cl^-, total CO2, and Glucose, good correlations (r-values 0.7858 - 0.9974) was observed when comparing plasma/serum GEM4000 samples to plasma/serum Modular P samples. Although many comparisons were demonstrated statistical significance, none of the parameter differences were found to be clinically significant (Table 1).

Conclusion: The GEM4000 BG analyzer is designed to be used on whole blood samples, but we found that its off-label use on plasma and serum samples produced blood electrolyte and metabolite measurements that were interchangeable with paired Roche Modular P lab analyzer results. The interchangeability of results therefore seems to be affected more by different sampling method effects than by different measuring methods.

Table 1. Correlation and parameter difference statistics for plasma and serum measurements between GEM4000 and Modular P. Differences are calculated as y – x.

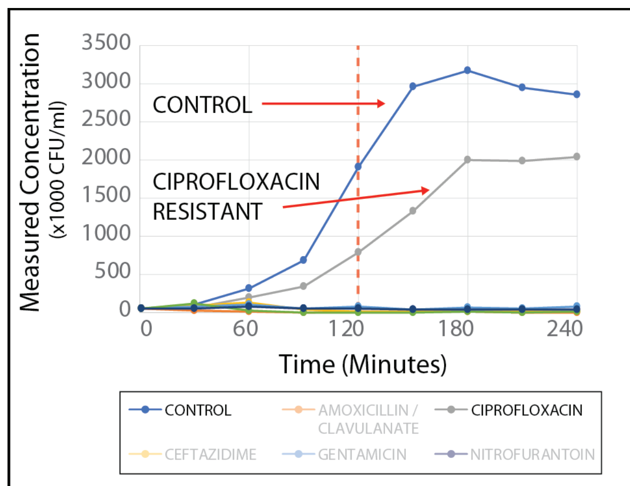
	GEM4000 plasma (y) vs. Modular P plasma (x)							GEM4000 serum (y) vs. Modular P serum (x)							
	Slope (95% CI)	Intercept (95% CI)	Corr. Coeff.	GEM4000 Median (IQR)	Modular Median (IQR)	Median diff.	p-value	Slope (95% CI)	Intercept (95% CI)	Corr. Coeff.	GEM4000 Median (IQR)	Modular Median (IQR)	Median diff.	p-value	Clinical acceptance limits (mmol/L)
Na^+	0.6370 (0.4685-0.8055)	51.5821 (27.7578-75.4064)	0.7858 (0.6331-0.8796)	141 (2.8)	141 (3.8)	0.5	0.1393	0.6142 (0.4653-0.7632)	54.1737 (33.2679-75.0795)	0.8088 (0.6694-0.8932)	140.5 (1.8)	141 (3)	0	0.7762	4
K^+	1.0502 (0.9923-1.1082)	-0.3597 (-0.5947--0.1248)	0.9889 (0.9792-0.9940)	3.8 (0.5)	4 (0.6)	-0.2	<0.05	1.0678 (1.0199-1.1158)	-0.3504 (-0.5585--0.1423)	0.9906 (0.9825-0.9950)	4.3 (0.5)	4.4 (0.6)	-0.1	<0.05	0.5
Cl^-	0.8299 (0.7448-0.9150)	19.7947 (10.8746-28.7148)	0.9522 (0.9123-0.9742)	107 (2)	105 (2)	2	<0.05	0.8596 (0.7533-0.9660)	16.4086 (5.2465-27.5706)	0.9467 (0.9025-0.9712)	107 (1.8)	105 (2.8)	2	<0.05	4
TCO_2	1.1446 (0.9182-1.3711)	-2.5049 (-9.2876-4.2778)	0.8402 (0.7202-0.9114)	31 (5)	29.5 (4)	2	<0.05	1.0721 (0.7894-1.3549)	-0.4855 (-9.1197-8.1487)	0.8007 (0.6566-0.8884)	32 (3)	30 (3)	2	<0.05	6
Glu	1.0682 (1.0315-1.1050)	-0.2318 (-0.4234--0.0402)	0.9959 (0.9923-0.9978)	5.3 (1.5)	5.2 (1.4)	0.1	<0.05	1.1102 (1.0731-1.1473)	-0.4515 (-0.6549--0.2482)	0.9974 (0.9952-0.9986)	5.3 (1.4)	5.1 (1.3)	0.2	<0.05	0.7

B-222

A Rapid, Point-of-Care Antibiotic Susceptibility Test for Urinary Tract Infections

T. J. Abram[1], M. Toosky[1], J. T. Grunwald[1], C. Ou[1], B. Shen[1], W. Zhao[2], E. Nicolai[3], D. Pala[3], M. Lera[3], G. Motolese[3]. [1]*Velox Biosystems, Irvine, CA,* [2]*UC Irvine, Irvine, CA,* [3]*ASI, Milan, Italy*

The rapid increase in antibiotic resistance in Urinary Tract Infections (UTI), due to the combination of high prevalence, high misdiagnosis rate, and the empirical use of antibiotics, poses a dire threat for future UTI treatment and management. With our direct bacteria counting technique, the objective of this study was to determine if changes in growth of a low-concentration bacterial population could be detected without enrichment, thus reducing the typical timeframe for urine culture AST from 2-3 days to 2 hours. A total of 66 "presumptive UTI-positive" urine samples from 3 clinical sites were tested against one or more antibiotics, resulting in 107 unique data points. Based on EUCAST guidelines, antibiotic MICs were used to determine the minimal bactericidal concentrations (MBC) for time-dependent antibiotics (Amoxicillin, Ceftazidime, Nitrofurantoin) and concentration-dependent antibiotics (Ciprofloxacin, Gentamicin). For each sample, a fixed volume of urine was inoculated into 3ml of growth media with or without MBC concentrations of antibiotics from the panel and incubated at 37°C between measurements. At 0, 1, 2, and 3-hour increments, 30 µl aliquots of culture were added to 3ml of isotonic solution with Sytox® Orange, a cell-impermeant nucleic acid dye. These samples were heated for 7 minutes at 80°C to allow influx of the fluorescent dye and then scanned on the Velox 3D-scanning fluorescent particle counter for 1 minute. By comparing results of the Velox AST instrument to clinical results from a standard Vitek® 2 AST instrument (BioMérieux), an overall categorical agreement (CA) of 93.5% was observed (sensitivity = 94.0%, specificity = 91.3%, N=107). Performance for individual drugs ranged from 86.4% CA (Gentamicin) to 100% (Amoxicillin). Overall categorical agreement increased from 66.0% to 93.5% and 97.0% for 1, 2, and 3-hour end-points, respectively, demonstrating that a rapid 2-hour AST test has clinical utility in high prevalence settings, such as UTI.



B-223

Evaluation of the Alere DETERMINE™ HIV-1/2 Ag/Ab Combo Kit Assay

B. Kelley, A. Wockenfus, L. Donato, B. Katzman, B. Karon. *Mayo Clinic, Rochester, MN*

Background: CDC guidelines now recommend a combined antibody/p24 antigen test for primary screening for HIV. We evaluated the Alere DETERMINE™ HIV-1/2 Ag/Ab Combo (Abbott Diagnostics) assay for rapid HIV testing using a combined antibody/antigen approach. **Methods:** Precision studies were carried out using both Alere DETERMINE™ HIV-1/2 Ag/Ab Combo Controls (five replicates per day over five days) and SeroDetect (Zeptomatrix Corporation) HIV-1/HIV-2 Ag/Ab Combo Verification Panel (two replicates per day over two days). Both products contained: Ag/Ab negative, HIV-1 Ab positive, HIV-2 Ab Positive, and a HIV-1 Ag positive controls. The SeroDetect panel also included a HIV-1 Ag/Ab positive control. Accuracy was assessed using a total of 81 tests using 60 residual samples (N=40 serum, N=20 plasma) previously analyzed by the reference method (Geenius HIV 1/2 Supplemental Assay, Bio-Rad). Accuracy for Ag testing was performed with 10 negative residual plasma samples spiked with SeroDetect controls (two samples spiked with negative control, three with HIV-1 Ag/Ab control, and five with HIV-1 Ag control). A WHO International Standard (Potters Bar) product was used to assess the limit of detection (3.0 IU/mL) for the HIV-1 p24 Ag reaction area. The WHO material was diluted from the original concentration of 1000 IU/mL to 3.0 IU/mL and 2.8 IU/mL. Six EDTA whole blood and plasma samples were used to compare readability of reaction lines. All studies were conducted blind, read in duplicate, and results were not shared between readers. **Results:** Precision/reproducibility experiments using both commercial control materials demonstrated 100% concordance (120/120) with expected results. Ab accuracy compared to the reference method was 98% (59/60 samples), of which 30 were HIV Ab positive. Ag accuracy was 100% (10/10) using spiked samples. One discordant Ab result was positive by the reference method and indeterminate on the Alere (reaction line in the Ab window but no control line). Upon sample dilution, both Ab and control lines were observed. It was noted that 10% of tests (8/81) gave weak/no control lines. First and second technologist result interpretations were 100% concordant (including those that gave weak/no control lines). Three technologists read the WHO Standard results and each recorded different results. At the 3.0 IU/mL concentration, Ag reactive results were observed by all readers. However, at the 2.8 IU/mL concentration no Ag reaction line was observed by any readers, but two readers saw an Ab reaction line where none was expected. Whole blood was observed to be less readable (5/12 tests had clear backgrounds) compared to plasma (11/12 clear backgrounds). Lastly, in multiple lots abnormal white "ghost" lines were observed in the control window of some test strips. **Conclusion:** Concordance between Alere DETERMINE™ HIV-1/2 Ag/Ab test and the reference method is high (98%). However, one discordant result using patient material which demonstrated no control line unless diluted was suggestive of high dose hook effect. The relatively high rate of weak or no control line observed (10%) as well as "ghost" lines in some lots could result in inaccurate interpretation. We therefore elected not to implement the Alere DETERMINE™ for rapid HIV screening.

B-224

Evaluation of the Correlation across Siemens Healthineers Blood Gas and Clinical Chemistry Systems for Electrolytes and Metabolites

C. Bethoney, S. Fennell, S. Chin, J. Tuck. *Siemens Healthineers, Norwood, MA*

Objective: The objective of the study is to demonstrate an end-to-end solution across the Siemens Healthineers three blood gas systems and a central laboratory-based analyser. The epoc® Blood Analysis System for patient-side testing, the RAPIDPoint® 500 Blood Gas System designed for the POC and the RAPIDLab® 1265 Blood Gas System, the laboratory-based system - and the central laboratory-based ADVIA® 1800 Clinical Chemistry system were evaluated for specific critical care analytes to demonstrate harmonization throughout the diagnostic product portfolio.

Relevance: The AACC's International Consortium for Harmonization of Clinical Laboratory Results continues in the effort to manage harmonization activities worldwide. The importance of harmonization of test results lies in the ability to obtain a result that leads to the same interpretation regardless of the measurement procedure, where and when the testing was done, or the units of measure. Harmonization assists in minimizing the misinterpretation of test results and the potential for adverse patient outcomes.

Methodology: A method comparison study was performed on the Siemens blood gas systems (epoc Blood Analysis System, RAPIDPoint 500 and RAPIDLab 1265 Blood Gas Systems) with whole blood versus the ADVIA 1800 Clinical Chemistry System with plasma. The study design and analysis followed CLSI EP09c for the measurement of sodium, potassium, chloride, glucose, and lactate. Correlation statistics including regression types, slopes, intercepts, and coefficients of determination (r^2) were generated.

Validation/Results:

Table 1. Method Comparison Statistics for Glucose Analyte: Siemens Healthineers Blood Gas Systems vs. ADVIA 1800 Clinical Chemistry System

Comparison	n	Bias at 45 mg/dL (MDL 1)	Bias at 120 mg/dL (MDL 2)	Bias at 180 mg/dL (MDL 3)	Deming Slope	Intercept	r^2	Interval mg/dL
epoc system vs. ADVIA 1800 system	40	1.56	7.19	11.70	1.08	-1.82	0.9948	27.7 to 617.35
RAPIDPoint 500 system vs. ADVIA 1800 system	40	1.31	8.34	13.96	1.09	-2.90	0.9888	27.7 to 617.35
RAPIDLab 1265 system vs. ADVIA 1800 system	40	1.98	6.81	10.68	1.06	-0.92	0.9898	27.7 to 617.35

Conclusion: Harmonization at the clinically relevant medical decision levels was demonstrated for a true end-to-end solution across the Siemens Healthineers blood gas and clinical chemistry systems for electrolytes and metabolites.

Note: Data for all analytes will be presented in the poster.

The products/features (mentioned herein) may not be commercially available in all countries. Due to regulatory reasons their future availability cannot be guaranteed.

B-225

Clozapine Close to Patient Testing with an Easy to Use Point of Care Analyser

A. Sumanth, D. Kozo, J. Courtney, N. Siegfried, R. Gill, M. Hilaire, R. Harney, A. Venkateswaran, J. Jarrah, K. DiStefano, S. Salamone, I. Baburina. *Saladax Biomedical, Inc., Bethlehem, PA*

Background-Aim: Adherence to clozapine therapy is critical to treatment outcomes for patients with major mental illness. Therapeutic drug monitoring of clozapine has been strongly recommended in guidelines. Measurement of clozapine blood levels can provide clinicians objective evidence to help avoid treatment failures. Point of care testing (POCT) provides immediate, on-site results during a patient visit to enable clinicians to personalize treatment plans. The aim of the study was to develop a clozapine POCT assay for whole blood (WB).

Methods: Reagents for CE marked MyCare® Psychiatry Clozapine Assay Kit, an automated homogenous immunoassay to measure clozapine levels, were modified for testing clozapine levels in WB with a POCT analyzer. The analyzer is a small, portable device capable of rapid testing in minutes in a POCT setting. Analytical performance of the POCT was evaluated using spiked WB samples using scale-up reagents on 20 analyzers. Effect of sample hematocrit (HCT) in the range of 35-52%, repeatability, Limit of Quantitation (LoQ) were evaluated using spiked WB samples. Recovery was evaluated with three levels of clozapine spiked in WB samples from three individual donors. Linearity was evaluated using 9 levels of spiked WB samples. Within-laboratory precision was evaluated over 5 days with controls. Clozapine levels in WB in over 100 patient samples were compared to plasma levels using the MyCare Clozapine automated assay on a Beckman Coulter® AU 480.

Results: The total testing time of the POCT assay was < 7 minutes. The LoQ and linearity of the POCT were equivalent to that of MyCare Clozapine Assay Kit, with a linear assay range of 68-1500 ng/mL. Repeatability coefficient of variation was ≤ 6% and within-laboratory precision was ≤ 12%. Recovery of clozapine was between 90-107%. HCT did not show any interference in the test: all recovery was within ± 15%. Method comparison to the fully automated reagents had correlation coefficients ≥ 0.90.

Conclusions: The clozapine point of care test demonstrated robust performance, allowing for a rapid (< 7 min), precise, sensitive and specific measurement of clozapine in capillary human whole blood samples.

B-226

Comparison of Bias between CoaguChek XS Test Strips Calibrated to WHO IRP rTF/16 vs rTF/09

L. A. Hartman, A. M. Wockenfus, B. R. Kelley, L. L. Sorenson, B. M. Katzman, B. S. Karon, L. J. Donato. *Mayo Clinic, Rochester, MN*

Background: On June 6th 2018, Roche Diagnostics issued a bulletin acknowledging possible positive bias in a new lot of testing strips for the CoaguChek XS Pro/Plus systems that was calibrated to a new WHO IRP standard (rTF/16). The inaccurately high results could lead to incorrect medication dosing or mismanagement of patients on warfarin. On October 31st 2018, a Class 1 FDA recall on the rTF/16-calibrated lot of strips was announced by Roche. Following the recall, Roche released a newly created lot of testing strips, calibrated back to the previous WHO standard rTF/09. It was unclear what bias existed between the CoaguChek results and the IL ACL TOP 500 using RecombiPlastin 2G reagent (Instrument Laboratories).

Methods: To assess the amount of bias in the recalled strip lot, we performed testing in parallel between CoaguChek XS strips and our central laboratory reference method using RecombiPlastin 2G reagent on the ACL TOP 500 (Instrumentation Laboratories). The recalled lot of strips (rTF/16) was tested on patients (N=44) being seen in our Coagulation Clinic for routine capillary point of care (POC) INR monitoring. Patients were asked to provide comparison venipuncture samples immediately after their POC test. Comparison data between capillary POC and laboratory reference analyzer were evaluated using linear regression, bias, and overall percent within acceptable tolerance. We defined acceptance criteria as: slope = 1.00+/-0.10, $R^2 \geq 0.90$, and ≥90% of POC results within +/- 0.5 when INR was <5.0 or +/-10% when INR was ≥ 5.0. Once the newly created rTF/09 lot was released, the same comparison process was followed with additional patients (N=23).

Results: An average positive bias of 0.31 was observed on the CoaguChek using the recalled rTF/16 lot (range: 0.8-7.8 INR). Linear regression was acceptable with slope of 1.08 and R^2 = 0.96. However, only 33/44 (75%) were within +/- 0.5 or 10%. An average bias of 0.03 was seen across the range of testing (1.9-7.3 INR) on the newly created lot of strips (rTF/09). The slope for the newly created strips was 0.99 with an R^2 of 0.98. All (100%) of POC vs. reference method results met the tolerance limit using lot rTF/09.

Conclusion: The testing strips of the recalled lot (rTF/16) were found to have much larger discrepancies in bias and percent tolerance than the recalibrated lot rTF/09, Therefore, the recalled lot of strips had a greater potential to result in incorrect dose adjustments in patients who routinely use CoaguChek Plus/Pro testing to monitor their warfarin therapy.

B-227

Molecular Point of Care Test- Implementation Guidance for Pharmacist

J. Phillips[1], C. Fantz[1], N. Gabbita[2], U. Cowen[2], D. Klepser[3]. [1]*Roche Diagnostics, Indianapolis, IN,* [2]*Roche Molecular Diagnostics, Pleasanton, CA,* [3]*3University of Nebraska Medical Center, Omaha, NE*

Background: Community pharmacy-based disease management programs that utilize point-of-care testing services are anticipated to increase due to patient demand and payer desire to reduce cost of patient care. Many of these test systems are waived under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) and can be

performed with limited regulatory oversight under a Certificate of Waiver (CW) from the Centers for Medicare & Medicaid Services. Operators are directed to the manufacture's package insert for the instructions, intended use, and product limitations. Findings from a CMS and state agency surveyors indicated that the majority of CW testing sites performed testing correctly and provided reliable service. However, in CW sites, most directors and testing personnel did not have formal laboratory training or testing experience (which is not required when utilizing waived test), there was a high turnover of personnel, and lapses in following manufacturers' instructions and instituting practices to ensure the quality of the testing were noted. The survey findings indicated that 12% of the CW sites surveyed did not have the current manufacturers' instructions available, and 21% of the sites surveyed did not check to be sure there had been no changes to the instructions. Currently only one certification program exists; however several certificate programs are available. The NACDS certificate program is about training in the development and implementation of clinical services that include CW, POCT tests. While it covers the elements in the CDC's Ready, Set, Test, and To Test or Not to Test it is not training on use of any given test or platform. Additionally, it is important to note that the training program is not intended to cover trouble shooting of individual platforms. The **Objective:** of this study was to assess if current training adequately prepared pharmacists in a multi-center study to successfully utilize a new to them testing system. The study utilized the cobas® Liat testing system (Roche Diagnostics, Inc.) with the cobas® Strep A and cobas® Influenza A/B tests. Test operators were pharmacists. All participating pharmacists completed the National Association of Chain Drug Stores (NACDS) Community Pharmacy-Based Point-of-Care Testing certificate program prior to implementation of the study. **Methods:** The type and number of reported questions/issues were compiled by the study managers; root causes were identified and documented when applicable. The study enrollment occurred from November 2016- April 2018. **Results:** Over the course of the study period, pharmacists successfully ran 242 tests. Pharmacy personnel reported 13 questions/issues encountered during testing. Study managers then divided the data into three categories based on the root cause identified: instrument error (15%), operator error (53.8%), and unknown (31%) with an average time to resolution being calculated at 4.8 days. A detailed account of questions/issues will be presented. **Conclusions:** The training program utilized was sufficient to prepare pharmacist to utilize molecular point of care test. The results from this study can be utilized to strengthen current POC training programs.

B-228

Evaluation of Point-of-Care Abbott Determine™ HIV 1/2 Antigen/Antibody Combo Assay in a Low HIV Prevalence Setting

E. Millen, A. Szeto, R. M. Rolan, Z. Zhao, R. Harris. *New York Presbyterian Hospital, New York, NY*

Background: Rapid detection of HIV infection is essential for the initiation of early treatment and patient management. The NY State Department of Health recommended HIV testing algorithm includes screening with an FDA approved antigen/antibody test kit that detects HIV-1 and HIV-2 antibodies and HIV-1 p24 antigen to test for established HIV-1 and HIV-2 infection and acute HIV-1 infection, respectively. The Abbott Determine™ HIV 1/2 antigen/antibody Combo assay is a 4th generation FDA approved rapid point of care test that is simple to perform and provides results in 20 minutes. The objective of this study was to validate this assay for implementation as a CLIA waived test in our institution in a low HIV prevalence setting.

Methods: A total of 76 individuals were included whose serum samples were tested using the Siemens ADVIA Centaur® HIV Ag/Ab combo assay. Samples selected included 18 non-reactive and 58 reactive, the latter including 28 samples that were close to the ADVIA cutoff. All 58 reactive serum samples were further tested with the Bio-Rad Geenius™ HIV 1/2 supplemental confirmatory assay to determine the accuracy of the results, making this the reference method. Of the 58 reactive samples by screening, 27 were negative, and 3 were indeterminate by the confirmatory method. Among these 76 individuals, 56 leftover serum samples (same samples for ADVIA testing) and 20 leftover venous EDTA whole blood samples (collected at the same time as the serum samples for ADVIA testing) were tested using the Abbot Determine HIV combo assay. The sensitivity and specificity of this method were calculated by comparing the results to the reference method. This study has been determined as a QI/QA procedure not required for IRB approval.

Results: The overall sensitivity and specificity of the Abbott Determine ™ HIV antigen/antibody combo assay was 100% and 88%, respectively, for an overall agreement of 92% with the reference method. The specificities of the whole blood and serum samples were 100% and 83%, respectively. The calculated sensitivity is consistent with the manufacturer's claims and with prior studies evaluating the assay's performance. The specificity using the venous whole blood was comparable to the manufacturer's claim (99.7%) while the specificity of serum samples was remarkably lower than the manufacturer's claim (99.6%). A small sample size and the inclusion of samples that were positive by the screening method but indeterminate by the reference method could explain this discrepancy. Exclusion of these samples in the analysis resulted in an improved specificity of serum samples to 93%.

Conclusion: In our laboratory with a low HIV prevalence setting, the Abbott Determine HIV™ antigen/antibody combo assay demonstrated a sensitivity of 100%, supporting its use as an acceptable screening test. The specificity using the venous whole blood samples was superior to that of the serum samples. Reactive results will require further testing with a confirmatory assay, addressing any potential limitations in specificity. Future studies using larger number size are warranted.

Wednesday, August 7, 2019

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

Proteins/Enzymes

B-229

Glucose-6-phosphate Dehydrogenase Mutant D306C has a Higher Inhibition Rate for Enzyme Multiplied Immunoassay of Cholyglycine

J. Gong, J. Qi, L. Xiao. *Beijing Strong Biotechnologies. Inc, beijjing, China*

Background: Since Rubenstein et al. pioneered an EMIT approach for homogeneous enzyme competitive immunoassay, the EMIT assays are now routinely used in clinical analysis. G6PDH from *Leuconostoc mesenteroides* is the most commonly used enzyme for EMIT assay. The principle of the assay is that analyte (e.g. Cholyglycine, CG) in the sample compete with CG labeled with G6PDH (G6PDH-CG conjugate) for antibody binding sites. G6PDH-CG conjugate has substantial enzyme activity when not bound to CG antibody but has dramatically reduced enzyme activity when bound to CG antibody. Therefore, a key requirement for EMIT CG assay is that G6PDH-CG conjugates give significant repression of enzyme activity upon antibody binding. Here we first describe a new G6PDH mutant D306C with increased inhibition rate ,compared with commonly used mutant A45C or K55C. Further, D306C has the best calibration curve when compared with commercially available EMIT CG kits. **Methods:** Wild-type G6PDH gene and mutant was synthesized by Genewiz (Suzhou, China) and cloned into expression vector pET28a (*Bam*H*I/NcoI*). CG antibody was purchased from Biomed (Tianjin, China). G6PDH-CG conjugates were synthesized in the reaction buffer (0.1M PB, 0.15M NaCl, pH 7.0) with a ratio of 30:1 (CG derivatives: G6PDH mutant) for 2 hours at room temperature. Then the conjugates were dialyzed against the reaction buffer to remove the unreacted CG derivatives. Hitachi 7180 (Japan) was used to measure the 340nm absorbance. **Results:** The inhibition rates of different mutants are presented in Table 1. The G6PDH mutant D306C has the highest inhibition rate, which is an advantage for the EMIT CG assay development, as the S6/S1 ratio is the best. **Conclusion:** We first describe a new G6PDH mutant, D306C, with improved inhibition rate (48%), compared with A45C (28%) and K55C(33%). The improvement of the inhibition rate contributes to a better calibration curve of the EMIT CG assay on Hitachi 7180.

Table 1. The 340nm absorbance change, inhibition rate and calibration data of different G6PDH-CG conjugates and two commercially available EMIT CG assay

		D306C	A45C	K55C	Competitor A **	Competitor B **
Inhibition*	$(\Delta Abs)_{340nm}$ with CG antibody	13500	13516	13474	NA	NA
	$(\Delta Abs)_{340nm}$ without CG antibody	25997	18848	19999	NA	NA
	inhibition rate	**48%**	**28%**	**33%**	**NA**	**NA**
Calibration*	$(\Delta Abs)_{340nm}$ of S1 (0 μg/ml)	6615	6614	6613	6617	6612
	$(\Delta Abs)_{340nm}$ of S2 (2.5 μg/ml)	8054	8105	7022	7894	7493
	$(\Delta Abs)_{340nm}$ of S3 (5 μg/ml)	8861	8733	7619	8386	7864
	$(\Delta Abs)_{340nm}$ of S4 (10 μg/ml)	10149	9199	8533	9069	8967
	$(\Delta Abs)_{340nm}$ of S5 (20 μg/ml)	11699	9537	9314	9903	9664
	$(\Delta Abs)_{340nm}$ of S6 (40 μg/ml)	13009	9726	9879	10617	10086
	S6/S1 ratio	**197%**	**147%**	**149%**	**160%**	**153%**

* The experiments were carried out on a Hitachi 7180 analyzer and the absorbance change of different G6PDH-CG conjugates were calculated between point 18 and point 23. R1: 0.1M Tris, 5mM NAD (nicotinamide-adenine dinucleotide), 5mM G6P(Glucose 6-phosphate), 0.5 U/ml CG antibody, pH 7.5; R2: 0.1M PB (Phosphate Buffered Saline), 0.05ng/ml G6PDH-CG conjugate, pH 8.0. S1 to S6 referes to CG calibrators with concentrations indicated.**Inhibition rate is not available for these two kits.

B-230

Liver Markers in Alcohol Dependence Syndrome Patients

M. Raut, B. K. Yadav, S. Ghimire, V. K. Sharma, A. Bhattarai. *Institute of Medicine, Kathmandu, Nepal*

Background: Alcohol dependence syndrome (ADS) has become a global public health challenge because of its high prevalence and the concomitant increase in risk of liver disease, cardiovascular disease and premature death. Influence of alcohol use on liver metabolism is well recognized. This study was aimed at examining the association of liver markers like bilirubin, serum albumin, γ−glutamyltransferase (GGT) and aminotransferase, with alcohol dependence syndrome patients.

Methods: This cross-sectional study was conducted in TU Teaching Hospital. ADS patients were screened by the consultant psychiatrist using the Alcohol Use Disorder Identification Test (AUDIT) questionnaire. A total of 89 patients scored positive on the AUDIT as having alcohol-related problems and were included in the study. Blood Pressure and other anthropometric parameters were measured while blood samples were analyzed for liver markers and other blood parameters. **Results:** Mean age of cases and controls was 35.42 ± 5.6 & 34.53 ± 3.5 years respectively. The mean values of Gamma GT (181.02 ± 78.16), Alkaline Phosphatase (219.93 ± 76.87), albumin (36.61 ± 5.5). The mean values for serum bilirubin (total as well as direct), SGOT and SGPT were elevated significantly in cases as compared to the controls (p less than 0.001). Among the ADS cases serum GGT level was elevated in 97% patients. The SGOT/SGPT ratio was also significantly higher in cases (2.02 ± 0.39) and control (1.45±0.62). It was found that 15.1 % cases had low serum protein level and 32.9% cases were low serum albumin level. Albumin to globulin ratio was also significantly decreased in cases (1.16 ±0.29). **Conclusion:** These findings support the hypothesis that, alcohol may affect the pattern of liver markers and also damage the liver cells. Decrease in serum albumin and elevation of SGOT to SGPT ratio more than two is suggestive of development of liver cirrhosis in alcohol dependence patients.

B-231

Diagnostic Accuracy of Kidney Injury Molecule 1 (KIM-1) for Predicting Diabetic Nephropathy in Type 2 Diabetic Patients: A systematic review and meta-analysis

G. V. Kapoula, P. I. Kontou, P. G. Bagos. *Department of Computer Science and Biomedical Informatics, School of Science, University of Thessaly, Lamia, Greece*

Background: Diabetic Nephropathy (DN), is a serious microvascular complication of diabetes mellitus resulting in end-stage renal disease with morbidity and mortality. At present, the diagnosis of DN is based on the abnormal concentrations of urinary albumin caused mainly by glomerular hyperfiltration, a condition known as microalbuminuria or incipient nephropathy. Its pathogenesis is multifactorial and it seems to be associated with both glomerular and tubular interstitial damage. In fact it has been shown that in the absence of glomerular proteinuria, tubular dysfunction can even proceed glomerular injury and thus, microalbuminuria. Hence, although microalbuminuria measurement remains the gold standardized, non-invasive test to predict the onset and monitor the progression of DN, its predictive value has been challenged by researchers which believe that is not as high as initially considered, leading to a shift to novel biomarkers which will help identify diabetic renal lesions early enough.

Kidney injury molecule 1 (KIM-1) is a type I transmembrane glycoprotein expressed on the renal proximal tubule epithelial cells mainly after ischemic injury. It has been also suggested to be a sensitive and specific marker of tubular damage in various renal diseases. The aim of this systematic review and meta-analysis was to assess the value of urinary KIM-1, as a biomarker for the diagnosis of early diabetic nephropathy (DN) in patients with type 2 diabetes mellitus (T2DM).

Methods: A comprehensive search was performed on PubMed by two reviewers until January 2019. Studies in which a) the degree of DN was determined according to the urinary albumin/creatinine ratio and b) urine KIM-1 normalized to creatinine was measured in healthy individuals and in T2DM patients with normoalbuminuria, were included in the meta-analysis. For each study, a 2x2 contingency table was formulated using as data the mean and sd, assuming they normally distributed. Sensitivity, specificity and other estimates of accuracy were calculated using bivariate random effects model. The hierarchical summary ROC (hsROC) method was used to pool data and to evaluate the area under curve (AUC). Heterogeneity was assessed using the I^2 test. Publication bias as assessed using Deeks test.

Results: The meta-analysis enrolled 5 studies involving 112 healthy individuals and 193 patients with type 2 diabetes. Overall, pooled sensitivity and specificity among the controls (healthy individual) and T2DM normoalbuminutric patients was 0.80 (95%CI; 0.40-0.96) with I^2 =95.23% and 0.80 (95%CI; 0.45-0.95) with I^2=91.03%, respectively. The positive likelihood ratio was 4.1 (95%CI 0.8-20.5), negative likelihood ratio was 0.25 (95%CI 0.05-1.38) and diagnostic odds ratio (DOR) was 16 (1, 426). The AUC of KIM-1 to distinguish controls from normoalbuminuric T2DM patients, was 0.87 (95%CI 0.84-0.90). There was no publication bias (p=0.4).

Conclusion: The results of this meta-analysis suggest that KIM-1 may be a possible early and good marker for predicting diabetic nephropathy in type 2 diabetic patients, supporting the finding that tubular dysfunction is a critical component of the early course of diabetic nephropathy.

B-232

Performance Evaluation of the Atellica CH Urinary/Cerebrospinal Fluid Protein (UCFP) Assay*

P. Datta, D. Louis, J. Dai. *Siemens Healthcare Diagnostics, Glasgow, DE*

Background: A Urinary/Cerebrospinal Fluid Protein (UCFP) assay (Siemens Healthineers) has recently been developed for quantitative determination of total protein in human urine and cerebrospinal fluid (CSF) on the Atellica® Chemistry (CH) Analyzer. The Atellica CH UCFP Assay has been evaluated with precision, linearity, interference, and method comparison studies.

Methods: The Atellica CH UCFP Assay uses a single ready-to-use reagent, a set of 5-level calibrators, and commercial controls. In this assay, a specimen is diluted and then reacted with pyrogallol red-molybdate reagents. The binding of the dye to a protein in the specimen results in increased absorbance at 596 nm. The analyte concentration of an unknown sample is determined from the calibration curve. The auto-dilution feature of the analyzer extends the assay analytical range by two-fold.

Results: The repeatability and within-lab CVs (80 replicates per sample) of the assay with two urine controls were 2.2% and 4.8% at 28.2 mg/dL and 0.9% and 2.1% at 70.6 mg/dL; with two CSF commercial controls were 1.4% and 2.8% at 45.6 mg/dL and 0.8% and 1.9% at 87.1 mg/dL; and with two urine pools were 2.9% and 6.0% at 21.2 mg/dL and 0.7% and 1.5% at 179.2 mg/dL, respectively. The assay had an analytical range/linearity of 6 mg/dL to 250 mg/dL (500 mg/dL with system auto-dilution). The assay (y) shows acceptable accuracy when compared with the UCFP assay on the Dimension® Integrated Chemistry System (x), also from Siemens Healthineers (both assays standardized to NIST reference material SRM 927), for both urine and CSF samples over the analytical range: y = 1.035x - 1.496 (r = 0.99, n = 118) for urine and y = 1.001x - 0.935 (r = 0.99, n = 109) for CSF. The assay has the same reference intervals as the Dimension UCFP Assay: urine <11.9 mg/dL and CSF 15-45 mg/dL. The assay did not show any interference (<10%) with 34 compounds and preservatives that may be present in urine and CSF samples. The assay has a minimum of 30 days of stability on-system and 14 days of calibration stability.

Conclusion: The Atellica CH UCFP Assay demonstrates acceptable precision and accuracy on the Atellica CH Analyzer.

*Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

B-233

Performance Evaluation of the ADVIA Chemistry Urinary/ Cerebrospinal Fluid Protein (UCFP) Assay*

P. Datta, P. Hickey, J. Dai. *Siemens Healthcare Diagnostics, Glasgow, DE*

Background: ADVIA® Chemistry Urinary/Cerebrospinal Fluid Protein (UCFP) Assay (Siemens Healthineers) has recently been developed for quantitative determination of total protein in human urine and cerebrospinal fluid (CSF). The ADVIA Chemistry UCFP Assay has been evaluated on the automated, random-access ADVIA® 1800, ADVIA® 2400, and ADVIA® Chemistry XPT Systems (Siemens Healthineers). The evaluation of this method included precision, linearity, and method comparison studies. **Methods:** All ADVIA Chemistry Systems use the same UCFP reagent packs, calibrators, and commercial controls. In this assay, a specimen is diluted and then reacted with pyrogallol red-molybdate reagents. The binding of the dye to a protein results in increased absorbance at 596 nm. By constructing a six-level standard curve (water is used as reagent blank) from the absorbances of five levels of standards, the analyte concentration of the sample is determined. **Results:** The repeatability and within-lab CVs (80 replicates per sample) of the method with two urine and two CSF commercial controls and two urine pools (protein concentration of ~20 to 180 mg/dL) on all ADVIA Chemistry Systems were <3.0% and <6.0%, respectively. The analytical range/linearity of the method on all ADVIA Chemistry Systems was from 6 mg/dL to 250 mg/dL. The method on the ADVIA 1800 system (y) show acceptable accuracy with the UCFP method on the Dimension® Integrated Chemistry System (x), also from Siemens Healthineers (both methods standardized to NIST reference material SRM 927), for both urine and CSF samples over the analytical range: y = 0.991x - 0.214 (r = 0.99, n = 118) for urine and y = 0.991x - 0.214 (r = 0.99, n = 109) for CSF. The ADVIA 2400 and ADVIA XPT UCFP methods, in turn, agreed with the ADVIA 1800 UCFP method (x). For urine samples with a range of 7.2-242.0 mg/dL, ADVIA 2400 = 1.042x - 0.977 (r = 0.99, n = 111) and ADVIA XPT = 1.035x - 1.028 (r = 0.99, n = 110). For CSF samples with a range of 13.7-236.1 mg/dL, ADVIA 2400 = 1.024x - 0.555 (r = 0.99, n = 119) and ADVIA XPT = 1.062x - 1.174 (r = 0.99, n = 119). The method did not show any interference (<10%) with 34 compounds and preservatives that may be present in urine and CSF samples. The method has a minimum of 30 days of stability on-system and 14 days of calibration stability. **Conclusion:** The ADVIA Chemistry UCFP assay demonstrates acceptable precision and accuracy on the ADVIA Chemistry Systems. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

B-234

Performance Evaluation of the Atellica CH CYSC_2 Assay

J. T. Snyder, J. Parker, J. Cheek, K. Kolewe, S. Janas, C. Theos. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH CYSC_2 Assay on the Atellica® CH Analyzer. Cystatin C (CYSC_2) is a cysteine proteinase inhibitor formed by all nucleated cells and is used as a marker of renal function. A reduction in the glomerular filtration rate causes a rise in CYSC_2 concentration. CYSC_2 is not dependent upon muscle mass or nutrition, factors that are known to affect creatinine values. The Atellica CH CYSC_2 Assay reagent is a suspension of latex particles coated with anti-cystatin C antibody. When patient sample containing cystatin C is mixed with reagent, agglutination occurs, resulting in increased turbidity.

Methods: Performance testing included precision, method comparison, interference testing, and detection capability. Assay precision was evaluated using CLSI guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3, with patient sample results compared with results from the ADVIA® Chemistry CYSC_2 assay on the ADVIA® 1800 Clinical Chemistry System. Interference testing was tested in accordance with CLSI EP07-A2. Detection capability was determined in accordance with CLSI EP17-A2.

Results: Within-lab precision ranged from 0.8 to 3.7% CV for serum and plasma samples. The method comparison study yielded a regression equation of y = 1.01x + 0.04 mg/L, with r of 0.999. CYSC_2 was tested at two different analyte concentrations for each interferent, and interference ranged from 0.6 to -5.8%. Detection capability testing included limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ). LoQ is the lowest amount of analyte that can be quantified with <10% CV. The Atellica CH CYSC_2 assay was determined to have an LoB of 0.08 mg/L, an LoD of 0.12 mg/L, and an LoQ of 0.25 mg/L.

Conclusion: The Atellica CH CYSC_2 Assay tested on the Atellica CH Analyzer demonstrated acceptable precision, interference testing, and detection capability. Method comparison results showed acceptable agreement compared to the ADVIA® Chemistry CYSC_2 assay.

B-235

Evaluation of a Latex Enhanced Immunoturbidimetric Assay Kit for the Rapid Direct On-Board Measurement of Glycated Haemoglobin (%HbA1c) on the RX daytona + Analyser

E. Donnelly, P. McGivern, E. Lenehan, M. L. Rodríguez, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: The glycated haemoglobin (%HbA1c) test is used to assess glycaemic control in people with diabetes. The level of %HbA1c increases proportionally to the level of glucose in the blood and is representative of the mean blood glucose level over the previous six to eight weeks. It is therefore, a long term indicator of diabetic control, whereas, blood glucose is a short term indicator. The availability of assay kits allowing rapid, accurate and reproducible measurement of %HbA1c facilitates long term monitoring of diabetes mellitus. This study reports the analytical evaluation of a liquid stable latex enhanced immunoturbidimetric assay kit with enhanced precision and accuracy for the rapid direct measurement of %HbA1c in human whole blood. The assay was applied to the fully automated benchtop analyser RX daytona + with sample pre-treatment being completed on-board the instrument.

Methods: The assay is based on latex immunoagglutination. HbA1c in the test sample is absorbed onto latex particles, and then cross-linked anti-HbA1c is added to form an antigen-antibody complex. Concentrations are calculated from a 5-point spline calibration curve. On-board and calibration stabilities were tested by storing the reagents uncapped on the RX daytona + for 28 days. Within-run and total precision were assessed by testing whole blood samples at defined medical decision levels, 2 replicates twice a day for 20 days. Correlation studies were conducted against the NGSP HPLC reference method and also an existing on-market assay. Whole blood patient samples (n >100) were tested.

Results: The HbA1c reagent presented an on-board stability of 28 days and calibration frequency of 28 days. The assay presented an assay range of 2.61 to 13.7 %HbA1c (top calibrator dependent). Within-run and total precision for three different concentration levels showed CV(%) values typically ≤5.0%. In the correlation study vs NGSP HPLC the following linear regression equation was achieved: Y = 0.966x + 0.168; r = 0.978. In the correlation study vs current on-market assay the following linear regression equation was achieved: Y = 1.000x + 0.043; r = 0.990.

Conclusion: Data indicates optimal analytical performance of this liquid stable latex enhanced immunoturbidimetric assay kit when applied to the benchtop analyser RX daytona +. The direct, on-board HbA1c testing capabilities of the system allows immediate testing, saving time. This fully automated application represents a rapid and reliable analytical tool for use in the determination of HbA1c in human whole blood.

B-236

Development of Tina-quant® C-Reactive Protein IV Assay for the Measurement of CRP in Serum and Plasma on Roche Clinical Chemistry Analyzers

A. Grebe[1], D. Pfleger[1], M. Schweiger[1], G. Papassotiriou[2], G. Trefz[1]. *[1]Roche Diagnostics GmbH Penzberg, Penzberg, Germany, [2]Diagnostics International Ltd., Rotkreuz, Rotkreuz, Switzerland*

Medical Background: CRP is the classic acute phase protein in inflammatory reactions. It is synthesized by the liver and consists of five identical polypeptide chains. CRP is the most sensitive of the acute phase reactants and its concentration increases rapidly during inflammatory processes. The CRP response frequently precedes clinical symptoms, including fever. The acute phase response develops in a wide range of acute and chronic inflammatory conditions like bacterial or fungal infections; rheumatic and other inflammatory diseases; malignancy and tissue injury or necrosis. During the acute phase response, levels of CRP rapidly increase within 2 hours of acute insult, increasing up to 50,000-fold in acute inflammation. With resolution of the acute phase response, CRP declines with a relatively short half-life of 18 hours. Due to its rapid rise in concentration in response to inflammation and infection, CRP is broadly used in diagnostics in screening for inflammation and infection.

Test Principle: Particle enhanced immunoturbidimetric assay.Human CRP agglutinates with latex particles coated with monoclonal antiCRP antibodies. The aggregates are determined turbidimetrically.

Development Goals - Development of an application for analysis of serum and plasma samples. - CRP reagent harmonisation on all Roche clinical chemistry analyzers. - Traceability against ERMDA474/IFCC. - High end of measuring range ≥ 350 mg/L (3332 nmol/L). **Results:** ***Traceability*** CRP4 has been standardized against the certified reference material ERMDA474/IFCC. ***Measuring range and lower limits of measurement*** The linear assay range is 0.6-350 mg/L (5.7-3332 nmol/L). Extended measuring range: 700 mg/L (6664 nmol/L). ***Lower limits of measurement***

Limit of Blank: 0.2 mg/L (1.9 nmol/L), Limit of Detection: 0.3 mg/L (2.9 nmol/L), Limit of Quantitation: 0.6 mg/L (5.7 nmol/L) with a Total Error of 20%. ***Limitations and Interferences:*** No interference of bilirubin (conjugated and non-conjugated) up to a I-Index of 60, of lipaemia (Intralipid) up to a L-Index of 1000, of hemoglobin up to a H-Index of 1000, Rheumatoid Factors (1200 IU/mL), Albumin (60 g/L) and Immunoglobulins (50 g/L) was observed. High dose hookeffect: No false result occurs up to a CRP concentration of 1200 mg/L (11424 nmol/L). ***Precision - CLSI EP5 - 21 days*** Three controls, five human sera, sample concentration range: 1.43-303 mg/L (13.6-2885 nmol/L): Total precision in all samples < 1.9 (%CV). ***Method Comparison Studies:*** Human serum samples obtained on a COBAS INTEGRA 400 plus analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi **cobas c** 501 analyzer (x) (n = 157). Passing/Bablok regression: y = 1.02x + 0.0949 mg/L (0.903 nmol/L). The sample concentrations were between 0.610 and 311mg/L (5.81 and 2961 nmol/L).

Comparison to CRP method on Siemens ProSpec: y = 1.01x - 0.212 mg/L (-2.02 nmol/L). **Conclusions:** The introduction of CRP4 assay will harmonize the CRP assay portfolio for screening of inflammation and infection. All of the development goals for the application of CRP4 assay on Roche clinical chemistry analyzers were met. The use of CRP4 assay will improve CRP testing in patient samples in the laboratory routine.

B-237

Validation of an Automated Glutamate Dehydrogenase Enzyme Activity Assay in Serum and EDTA Plasma

S. Kim, H. Kincaid. *Covance, Los Angeles, CA*

Background: Hepatotoxicity is an ongoing focus for drug development. Glutamate dehydrogenase (GLDH) is recognized as an important biomarker for monitoring liver damage in patients enrolled in clinical trials. Drug-induced liver injury (DILI) figures prominently in the termination of candidates for new drug applications (NDA) to the FDA and other regulatory authorities. Recently, pharmaceutical companies are requesting GLDH in their clinical trial protocols. In response, the Randox GLDH assay was validated in both serum and EDTA plasma.

Methods: The Randox GLDH assay is intended for the quantitative determination of GLDH activity (CE marking, RUO in US) in serum. Samples are incubated at 37 °C with NADH, ammonium, and α-oxoglutarate. As NADH is oxidized by GLDH, the decrease in the absorbance is measured spectrophotometrically at 340 nm and is proportional to GLDH activity. Serum studies were conducted on a Roche Cobas® 8000; plasma studies conducted on a Beckman Coulter AU400 chemistry analyzer. Data analysis utilized EP Evaluator (version 9.4 or greater).

Results: Serum measurements covered the AMR (3-90 U/L) defined by Randox. Recovery of five concentrations of commercial quality control material defined the linear response (bias ≤6.1%; slope = 0.978; intercept = 0.16). Accuracy was defined as the recovery of assigned target values of 2 commercial QC materials (achieved ≤4.7%). The CV for within-run precision (n=20) was ≤3.4% and for between-run precision (n=100, 10 days) was ≤5.2%. The study defining the estimated LLOQ included 8 concentrations (n=240, 8 days) resulting in 2.4 U/L with a CV of ≤20%. Dilutions up to 1:16 defined an ULOQ of 1440 U/L. The reference interval (<3 to 8.9 U/L) was verified with 60 samples (30 male, 30 female), confirming the reference interval of ≤10 U/L reported in the literature (Schomaker, et al. 2013). Stability studies (mean recovery ≤20%) were performed under the following conditions: ≤4 days at 2-8 °C, ≤1 day at ambient temperature, and ≤7 days at -20 and -70 °C. Stability was acceptable after 5 freeze-thaw cycles

Plasma (K2EDTA) measurements on the Beckman Coulter AU400 covered the AMR (3-50 U/L) defined by Randox. Recovery of five concentrations of pooled plasma defined the linear response (bias ≤6.4%; slope = 1.031; intercept = 0.003). The reference interval in plasma was verified with 120 samples and found to be comparable with serum: 117 of 120 (97.5%) samples were ≤8.9 U/L. A serum/plasma acceptable correlation was established with 30 paired samples (R = 0.9997; bias = -5.3%; slope = 0.955, intercept = -0.199). Stability studies (mean recovery ≤20%) were performed under the following conditions: ≤3 days at 2-8 °C, ≤3 days at ambient temperature, and ≤7 days at -20 and -70 °C. Stability was acceptable after 5 freeze-thaw cycles.

Conclusion: This data demonstrates that the Randox GLDH assay is valuable and robust in the diagnostic assessment of hepatotoxicity in clinical trials.

B-238

Frequency of Serum Free Light Chain Abnormalities in Patients with High Levels of Serum Paraproteinemia Detected by Cappilary Eletrophoresis

G. Loureiro, M. Benini, N. Shaaban, M. Martins, M. Cavalheiro, A. Cain, J. de Sá, N. Sumita. *Fleury, São Paulo, Brazil*

Background: Concentration of serum free light chains (sFLC) has been promoted as an assay in the screening and evaluation of monoclonal gammopathic manifestations in addition to serum protein electrophoresis (SPEP) and immunofixation (IFE). The majority of patients with intact immunoglobulin multiple myeloma (MM) present abnormal serum free light chain ratio. However, about 5 % percent of patients may present high concentration of M-component with normal sFLC ratio. **Objective:** To assess the frequency of normal and abnormal sFLC ratio in samples with high levels of M-protein detectable by capillary serum electrophoresis in a large sample from a private laboratory in Latin America. **Methods:** During a 12 month period (January to December, 2018) all samples with had SPEP and sFLC ordered in conjunction were analyzed. SPEP was analyzed by capillary electrophoresis method (Sebia, France) and sFLC by immunoturbidimetric method (The Biding Site, UK). Abnormal sFLC ratio was considered when outside the reference range (0.26-1.65) and a high level of M-component was considered when serum concentration superior to 3 g/dL. Results were expressed as a frequency percentage and mean±standard deviation. **Results:** A total 142,876 SPEPs were ordered and 3,135 had sFLC ordered in conjunction. Of these samples, 103 samples had quantifiable M-component on SPEP superior to 3 g/dL (4.56±1.26). Of those samples, 99 had abnormal sFLC ratio (419.08±646.27 when

Kappa involvement and 0.05±0.05 when Lambda involvement) and 6 had a normal sFLC ratio (1.06±0.45). Patients with normal sFLC, 4 had identifiable IgG Kappa, 1 IgG Lambda and 1 IgM Kappa. **Conclusion:** Our results support literature findings of low frequency normal sFLC ratio in patients with high levels of paraproteinemia, which are likely to have a intact immunoglobulin MM diagnosis.

B-239

Falsely Decreased IgM Paraprotein on Capillary Electrophoresis Due to Protein Aggregation

Z. Su, A. Malone, P. Holmes. *Christiana Care Health System, Newark, DE*

Background: A blood sample from an 81-year-old man with a clinical history of anemia, back pain, hearing loss and lymphoma was sent to the lab for protein electrophoresis (PEP). A discrepancy of albumin quantification was observed between capillary electrophoresis (CE) and chemistry analyzer (5.9 g/dL *vs* 4.1 g/mL). Two small monoclonal peaks were observed in the beta region on PEP (Figure 1) and further Immunotyping confirmed them as IgM kappa monoclonal protein (Figure 2). However, the quantification of the monoclonal peak on CE was not consistent with the IgM measured on nephelometry (640 mg/dL vs 3300 mg/dL).

Methods: The sample was pretreated with different procedures to solve the protein aggregation before protein electrophoresis: 1) sample was warmed to 37 ºC; 2) sample was mixed with Fluidil, a dissociation agent provided by the manufacture; 3) sample was treated with 1% beta-mercaptoethanol (BME) solution.

Results: Neither warming nor mixing with Fluidil changed the CE results significantly. However, a large band in the beta region was observed on the electropherogram for the sample being treated with BME (Figure 3). Albumin and IgM values obtained on this electropherogram are consistent with those measured on chemistry analyzers (Alb: 4.0 vs 4.1 g/mL; IgM: 2700 vs 3300 mg/dL).

Discussion: The quantification of monoclonal proteins plays an important role in evaluating patients' response to treatment. Two main methods, protein electrophoresis and immunoassays performed on automatic analyzers (including nephelometric assay or turbidimetric assay), are used in clinical laboratories. The former is labor intensive but it can distinguish the disease-associated M- protein from background immunoglobulins. Generally, immunoassays are vulnerable to over-estimation of the paraproteins. Some monoclonal proteins, especially IgM, have a tendency to aggregate or form immuno complexes, which can result in aberrant quantification of paraprotein or multiple M-peaks. Some IgM aggregation due to cryoglobulinemia can be dissolved by warming the sample to 37 ºC. For many immunoglobulin aggregates, their higher order structures are stabilized by hydrophobic interactions and can be dissociated with a chaotropic agent, such as Fluidil provided by the manufacture. However, the aggregates held by disulfide bonds (S-S) cannot be dissociated by chaotropic agent, and instead, reducing agents, such as beta-mercaptoethanol, should be used.

References: 1)Murray DL, Ryu E, Snyder MR, et al. Quantification of Serum monoclonal proteins: relationship between agarose gel electrophoresis and immunonephelometry. *Clin. Chem.* 2009;55:1523-9.

2) Sheng ZM, Przygodzki RM and O'Leary TJ. 2-mercaptoethanol treatment improves measurement of an IgM M-protein by capillary electrophoresis. *Clin. Chem.* 2001;47:1326-7.

3) Uljon SN, Treon SP, Tripsas CK, Lindeman NI. Challenges with serum protein electrophoresis in assessing progression and clinical response in patients with Waldenström macroglobulinemia. *Clin Lymphoma Myeloma Leuk.* 2013;13(2):247-9.

Figure 1. Two small monoclonal peaks were observed in beta

region on PEP.

Figure 2.Immunotyping identified IgM Kappa monoclonal protein.

Figure 3. Comparable monoclonal protein was observed after the specimen was treated with beta-mercaptoethanol.

B-240

Cryoglobulin Testing in Clinical Laboratories

Z. Su, J. Matarese, M. Fitzpatrick, P. Holmes. *Christiana Care Health System, Newark, DE*

Background: Cryoglobulins are special serum immunoglobulins that precipitate at temperatures less than normal body temperature. Three groups of cryoglobulins can be found in patients: 1) monoclonal immunoglobulins such as IgM. This type of cryoglobulin usually has poor solubility at low temperatures and can cause problems with sample probes of automated analyzers; 2) immunoglobulins mixed with monoclonal components; and 3) immunoglobulins without monoclonal components[1,2]. Studies indicate that the majority of cryoglobulins are immune complexes containing rheumatoid factor (RF) that can be found in patients with viral infections (such as HCV and HBV), autoimmune diseases, vasculitis, and/or neuropathy[3].

Methods: The Christiana Hospital clinical laboratory developed and validated qualitative and quantitative assays for cryoglobulins. Briefly, after the samples are drawn from the patient, they must incubate at 37ºC for an hour. The serum is then separated from the red blood cells into three aliquot tubes and one tube is kept at each of the following temperatures: refrigerated (4ºC), room temperature (25ºC) and incubated (37ºC). Visual screening is performed by an MLS daily for seven days and a preliminary report is issued after the reading on the third day. On the seventh day, specimens are visually observed again and if turbidity exists, the specimen is treated following our procedure and then quantitative immunoglobulin (IgG, IgA, IgM) testing will be performed. The difference of total immunoglobulin between the 37ºC sample and the 4ºC sample is then calculated. Cryoglobulins are considered as present/positive if the difference is >0.4. In this study, the last two years (2017-2019) of test results have been retrospectively analyzed.

Results: A total of 305 cryoglobulins were tested in the past two years and positive results were obtained from 70 patients (23%). Among them, 35 were male and 35 were female, with an average age of 56 (range: 25-84). The majority of cryoglobulins were found in patients with HCV infections (31.4%, with 13 males and 9 females), autoimmune diseases (21.4% with 5 males and 10 females), and lymphoproliferative disorders (10% with 7 males and 1 female). Cryoglobulins were also found in other disease states: viral infections other than HCV (7.1%), bacterial infections (5.7%), neuropathy (5.7%), liver cirrhosis (2.9%), kidney diseases (2.9%), irritable bowel syndrome (1.4%), and unknown chronic inflammation (11.4%). Among the 70 patients with positive results, 20 were ordered for protein electrophoresis and monoclonal proteins were identified in five patients (25%). Further immunotyping proved that they were: two IgM kappa, one IgG kappa, one IgA lambda, and one IgM kappa plus IgA lambda.

Conclusions: The analysis of cryoglobulin in clinical laboratories plays an important role not only for the diagnosis of disease, but also can be helpful in identifying a potential interfering substance on automated laboratory analyzers.

References:

1)Hortin GL. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. Fifth Edition.

2) Shihabi ZK. Cryoglobulins: An Important but Neglected Clinical Test. Annals of Clinical & Laboratory Science. 2006;36: 395-408.

3) Kllemuchikkal U, Gorevic PD. Evaluation of Cryoglobulins. Arch Pathol Lab Med 1999;123:119-125.

B-242

Measuring Lysosomal Acid Lipase in Dried Blood Spots: A Reference Laboratory Experience

A. Perry[1], T. Yuzyuk[2], I. De Biase[2]. [1]*ARUP Laboratories, Salt Lake City, UT,* [2]*University of Utah; ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT*

Background: Lysosomal acid lipase deficiency (LAL-D; OMIM #278000) is an autosomal recessive lysosomal storage disease that can present at any age with hepatomegaly, elevated transaminases and dyslipidemia, progressing to liver fibrosis/cirrhosis and premature atherosclerosis. Symptoms are non-specific and overlap with several other genetic and non-genetic conditions. LAL-D is rare (estimated prevalence1: 130,000-300,000), but correctly diagnosing these patients is critical, since enzyme replacement therapy improves clinical outcome. A simple and relatively inexpensive diagnostic test is available that measures LAL activity in dried blood spots (DBS). In 2015, we have implemented this enzymatic assay in our reference laboratory. **Methods:** LAL activity is measured using the fluorimetric substrate 4-methylumbelliferyl (4mU) palmitate with the activator cardiolipin. The inhibitor Lalistat 2 discriminates between LAL and interfering lipases in DBS. We employed a 96-well plate format (wavelength: emission 441nm/excitation 326 nm). Recently, we modified the assay to eliminate mercuric chloride, which is highly toxic, as stopping reagent. DBS obtained by direct puncture or spotted using whole blood collected in EDTA, Acid Citrate Dextrose (ACD) or Sodium Heparin were validated. **Results:** LAL activity measurements are linear within a 0.05-2.99 nmol/hr/punch range. Within-run imprecision evaluated in samples with normal or low LAL activity was ≤15%. The between-run imprecision evaluated by analyzing a normal control with each clinical run was ≤20% (n = 44). No difference in analytical performance was noted eliminating the stopping buffer. Since implementing this assay (July 2015), 819 samples were tested from 794 patients, ranging in age from 8 days to 81 years (435 males, 356 females, 3 unknown). 56% of patients were adults (≥18 years of age) and only 2.5% were infants (≤1 year of age). In

the majority of samples (89.9%), LAL activity was within the normal range (0.50-2.3 nmol/hr/punch). An enzymatic activity above the normal range was present in 3% of samples. Although high values have no clinical significance, they can be an artifact of an oversaturated filter paper and recollection was recommended. Out of the remaining 58 samples displaying reduced LAL activity, the majority was within published carrier range (0.15-0.5 nmol/hr/punch). In few cases (n = 4), this was due to a compromised sample; upon repeat testing on a freshly recollected sample, LAL activity normalized. Only four out of 794 patients displayed levels below <0.05 nmol/hr/punch (<4% normal activity) consistent with LAL-D. In two of them further molecular testing confirmed the diagnosis. Follow-up testing was recommended for two other patients. **Conclusion:** Measurement of LAL enzymatic activity in DBS is a simple, robust and relatively inexpensive diagnostic test. Given rarity of the disorder, we diagnosed LAL-D only in four patients in 4 years (total n= 794). However, availability of treatment warrants exclusion of LAL-D in patients of all ages presenting with unexplained hepatomegaly, impaired liver function and/or dyslipidemia.

B-243

Unusual Patterns of Interferon-Gamma (IFN-γ) Measurements and TB1/ TB2 Discordant Results with QuantFERON-TB Gold Plus (QFT-Plus)

L. Rodrigues, V. Frade, C. Khawali, P. Udiloff, L. C. Pierrotti, L. Faro. *DASA, São Paulo, Brazil*

Background: QuantFERON-TB Gold Plus (QFT®-Plus) is widely used to determinate *Mycobacterium tuberculosis* (MTb) infection. The test is based on measurement of IFN-γ released from stimulated peripheral blood mononuclear cells with specific MTb antigens and positive results are interpreted as previous MTb exposure. The aim of this study is described the frequency and the patterns of QFT®-Plus unusual results and TB1/TB2 discordant results. **Methods:** QFT®-Plus is performed according to the manufacture's instructions (QIAGEN). The assay is compound by four blood tubes: 1) stimulated with long peptides measuring CD4 T-cell response (TB1); 2) stimulated with long and short peptides measuring CD4 and CD8 T-cell response (TB2); 3) unstimulated tube (negative control - Nil); and 4) stimulated with mitogen (positive control - Mitogen). QFT positive results is determined by he amount of IFN-γ release in TB1 and/or TB2 above 0.35 UI/ml. Unusual patterns were defined as: **High Nil** (FN-γ measurement in plasma of Nil control tube is higher than 8.0 IU/ml); **Low Mitogen** (IFN-γ measurement in plasma of Mitogen control tube is lower than 0.5 UI/ml); and **Discordant Antigen Responses** (IFN-γ measurement in plasma of TB1/ TB2 tubes subtracting IFN-γ measurement detected in Nil controls are discordant). **Results:** A total 245 QFT®-Plus tests were performed from Jan to Mar/2018. The majority of results were negative (166, 67.8%); 67 (27.3%) were positive and 12 (4.9%) indeterminate. Table 1 summarized the unusual and discordant results. **Conclusions:** Low mitogen was the most frequent unusual results. Discordant TB1/TB2 results are not frequent, but these results have been expected since TB1 and TB2 antigens responses are complementary. TB2 IFN-γ responses probably represent a more recent infection; in contrast, TB1 response represents a more remote MTb exposure. Therefore, the use of both antigens (TB1/TB2) tubes' results has significantly increased the sensitivity of QFT®-Plus test.

Table 1. Frequency of QuantFERON®-TB Gold Plus unusual results and TB1/TB2 discordant results (N=xx)

QFT®-Plus Tests	N	%
No unusual or discordant results	**222**	**(90.6%)**
With unusual results	**10**	**4.1%**
High Nil	2	0.8%
Low Mitogen	8	3.3%
Discordant antigen results	**13**	**(5.3%)**
Positive TB1/ NegativeTB2	3	(1.2%)
Negative TB1/ Positive TB2	10	(4.1%)

B-244

Do We Still Need Amylase in the Management of Acute Pancreatitis?

C. D. Furey, J. Buxbaum, A. B. Chambliss. *University of Southern California, Los Angeles, CA*

Background: Many organizations, including the Choosing Wisely® initiative, encourage the use of lipase over amylase in the diagnosis of acute pancreatitis due to its improved sensitivity and specificity and prolonged elevation, fostering a wider diagnostic window. However, many clinical laboratories still offer amylase testing, and thus both amylase and lipase are sometimes ordered concurrently and repeatedly for some patients. This study sought to evaluate ordering patterns and to compare utility of amylase and lipase in patients with acute pancreatitis.

Methods: We analyzed consecutive patients with acute pancreatitis admitted to LAC+USC Medical Center, a large, tertiary, academic public hospital, between March 2015 and March 2017. Patients were included in the cohort if they met two of the three criteria: lipase greater than three times the upper reference limit (URL), characteristic epigastric pain, or cross-sectional imaging consistent with acute pancreatitis. Data collection included pancreatitis etiology and severity, biochemical profiles of amylase and lipase, and clinical course. Amylase and lipase were measured on a Roche Modular P chemistry analyzer, with reference ranges of 30-130 U/L for amylase and 7-60 U/L for lipase. This study was approved by the Institutional Review Board of the University of Southern California.

Results: The study included 492 patients with mean age 45 (±15) years, and 51% were male. All patients had at least one lipase order during the admission, and only 51 patients (10%) had at least one amylase order. Initial lipase was significantly more elevated above URL than amylase (32.8x URL vs. 7.3x URL, p=0.0028). Of the patients with at least one amylase order, amylase was ordered only once for 31 patients (61%), twice for 8 patients (16%), and three or more times for 12 patients (24%). Of the patients with at least one amylase order, the etiology of the pancreatitis was defined as: 34 (67%) gallstone-related, 10 (20%) alcohol-related, and 7 (14%) other etiology. Of the 34 gallstone-related patients, 24 (71%) had laparoscopic cholecystectomy performed during the admission, which is ideally to be performed as early as possible when pancreatitis resolves, though no standard timeline definition exists for performing this procedure.

The average elapsed time from admission to laparoscopic cholecystectomy was 5.3 (±2.9) days. For the 34 gallstone pancreatitis patients for whom amylase was ordered, average time for amylase normalization to below 3x URL was 2.5 days, compared to 1.7 days for lipase (no significance, p=0.1868). Lipase normalized faster for 42% of gallstone pancreatitis cases, amylase normalized faster for 32% of cases, and the remainder exhibited normalization of amylase and lipase with the same blood draw time.

Conclusion: Compared to amylase, lipase was elevated more significantly above the URL upon initial result, and amylase measurement was not necessary in the diagnosis and management of 90% of patients with acute pancreatitis. However, co-ordering of amylase and lipase may have utility in more rapidly recognizing patient recovery for some gallstone pancreatitis patients, thus possibly allowing for more rapid initiation of laparoscopic cholecystectomy and reduced hospital admissions.

B-245

Development of Monoclonal Antibodies to Chromogranin A, A Biomarker for Neuroendocrine Dysfunction

H. Stott, P. Ratcliffe, H. Winter, J. W. Curry, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: Chromogranin A (CgA) is a member of the chromogranin family. Granins are acidic proteins predominantly localised in secretory granules of neuroendocrine cells. CgA promotes the generation of secretory granules, such as those containing insulin in pancreatic islet beta cells. CgA is also a precursor to other biologically active proteins; enzymatic cleavage at multiple sites enables tissue-specific proteolytic processing to generate vasostatin I and 2 (beta granin), pancreastatin, catestatin and others. These peptides also act as modulators of the neuroendocrine system. The serum concentrations of CgA are increased in neuroendocrine tumours (NETs). NETs constitute a heterogeneous group of rare tumours and clinical presentation of these is heterogeneous and non-specific. Serum levels of CgA are also increased in cases with pancreatic and prostate cancer. In addition, CgA is also increased in the circulation for other conditions such as renal and liver failure.The aim of this study was to develop monoclonal antibodies (mAbs) with the potential to be used in the development of efficient immunoassay-based methodologies for the detection of CgA, for application to clinical investigation. **Methods:** Sheep were immunized with recombinant mam-

malian fragments of chromogranin A. Lymphocytes B were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific antibody using enzyme-linked immunosorbent assay (ELISA) based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by direct binding ELISA to determine their specificity for CgA and cross reactivity with human secretogranin 3 protein (HS3P), CD37/CDC37A and prefoldin subunit 1 (PFDN1). Epitope binning assays were then conducted using biolayer interferometry. One representative sample from each epitope bin was tested by western blot for reactivity with recombinant full length human CgA, the N-terminal fragment of human CgA, human vasostatin 2 (a smaller N terminal fragment of human CgA) and bovine adrenal tissue lysates. **Results:** The preliminary ELISAs showed that the developed mAbs panel was target specific, presenting minimal cross-reactivity (mostly <1 % under conditions tested) with HS3P, CD37/CDC37A or PFDN1. The epitope binning assays allowed the subcategorization of five distinct epitope bins. One antibody from each of these epitope bins was assessed by western blot and it was observed that from these five antibodies, all of them detected, as a minimum, the recombinant N-terminal fragment of CgA and full-length protein. Three also recognized vasostatin 2, a smaller N-terminal fragment of CgA. In addition, four antibodies detected bands at a minimum corresponding to full length native CgA in bovine adrenal tissue lysate.

Conclusion: The results indicate that the developed mAbs are specific to CgA. The assessment of reactivity of five mAbs from distinct epitope bins, show detection of the recombinant N-terminal fragment of human CgA and full-length human CgA protein (5 mAbs) and detection of human Vasostatin 2 (3 mAbs). Four mAbs also detected full length CgA in bovine tissue lysate. These antibodies can then be used in the development of immunoassay-based technologies for the detection of CgA in test settings.

B-246

Reference Intervals of Apolipoprotein E in Healthy Chinese Han Adults

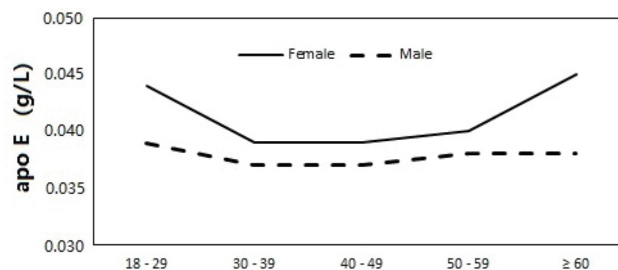
Y. Zhao[1], G. Feng[2], Y. Dai[3], Z. Liu[1], L. Feng[1]. [1]*The First Affiliated Hospital, College of Medicine, Zhejiang U, Hangzhou, China,* [2]*Women's Hospital, School of Medicine, Zhejiang University, Hangzhou, China,* [3]*Hangzhou Medical College, Hangzhou, China*

Background: Reference intervals (RIs) of Apo E levels are an important parameter for the clinical evaluation of patient health and the RIs of serum Apo E could be variable in different population. We plan to establish RIs of apolipoprotein E (Apo E) according to the CLSI EP28-A3 guideline in healthy Chinese Han adults.

Methods: Serum Apo E values were measured by immunoturbidimetry.The relationship between Apo E and age was analyzed by using Spearman correlation. The differences between the gender and age groups were compared using mann-whitney U test/ kruskal-wallis H test. We calculated recommended nonparametric $Q_{2.5}$ and $Q_{97.5}$ percentile intervals and the 90% confidence intervals (CI) of lower and upper limits to define the age- and gender- related RIs.

Results:1206 participants were divided into different groups according to gender and age (18-29, 30-39, 40-49, 50-59, and≥60 years). The level of Apo E was higher in females than males. Apo E was significantly associated with aging in adult females (r=0.108, P<0.05), but not in males (P=0.518). Thus serum Apo E levels among males in the five age groups were combined into one group. However, female Apo E levels showed statistically significant difference among the five groups (P = 0.020), then we found there were no statistically significant differences among these three groups:30 - 39, 40 - 49, and 50-59 years, and thus female participants were combined into three groups (18-29, 30-59, and≥60 years). Figure 1 also showed the trend of age-related medians of Apo E concentration fluctuated very little for males and fluctuated greatly for females.The RIs of Apo E for females were 0.0268-0.0619, 0.0247-0.0603, and 0.0269-0.0658 g/L for 18-29, 30-59, and≥60 years old respectively, that for males was 0.0242-0.0579 g/L.

Conclusion: Our results established the age- and gender-specific RIs of serum Apo E in healthy Chinese Han adults in our laboratory.



B-247

Validation of an Automated Enzymatic Assay for Quantitation of Glucose-6-Phosphate Dehydrogenase Activity in Whole Blood

X. Zhang, S. Zilka, X. Tang, J. Colon-Franco. *Cleveland Clinic, Cleveland, OH*

Background: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is an X-linked genetic disorder affecting NADPH generation which is essential for protecting red blood cells from oxidative injury. The severity of clinical manifestations, which include neonatal jaundice and hemolysis, is determined by the degree of enzyme deficiency. Most individuals with G6PD deficiency are asymptomatic and lack hematological alterations. However, medications, certain foods or infections can trigger hemolysis and hemolytic anemia. G6PD activity testing is important to identify deficiency, classify genetic variants and facilitate patient management. Tests for G6PD enzymatic activity are not commonly available for routine laboratory analyzers, limiting its widespread use across hospital laboratories. Here, we evaluated the performance characteristics of a G6PD reagent set on an automated analyzer.

Methods: Whole blood samples from 40 healthy donors and 150 patients were used. G6PD activity was determined by measuring the rate of NADPH formation at 340 nm using reagents form Pointe Scientific in a Cobas c501 analyzer (Roche Diagnostics). G6PD activity was expressed as Unit per gram hemoglobin which was measured using Sysmex KX-21 analyzer. The assay precision, analytical measurement range (AMR) and linearity were evaluated. Accuracy was determined relative to an established method at a reference laboratory. Data was analyzed using EP Evaluator software.

Results: The assay showed acceptable performance (Figure 1). The mean bias was less than 5% relative to the comparative method. The overall agreement in result interpretation (normal versus deficient) was 89.3%. All the discordant results (16 out of 150) were borderline samples with G6PD activity 8.6- 12.3 U/gHb.

Conclusion: This assay provides a simple and accurate method for quantitation of G6PD activity and diagnosis of G6PD deficiency. Additional clinical studies are needed to further refine the cutoff level for deficiency and better interpretation of borderline results.

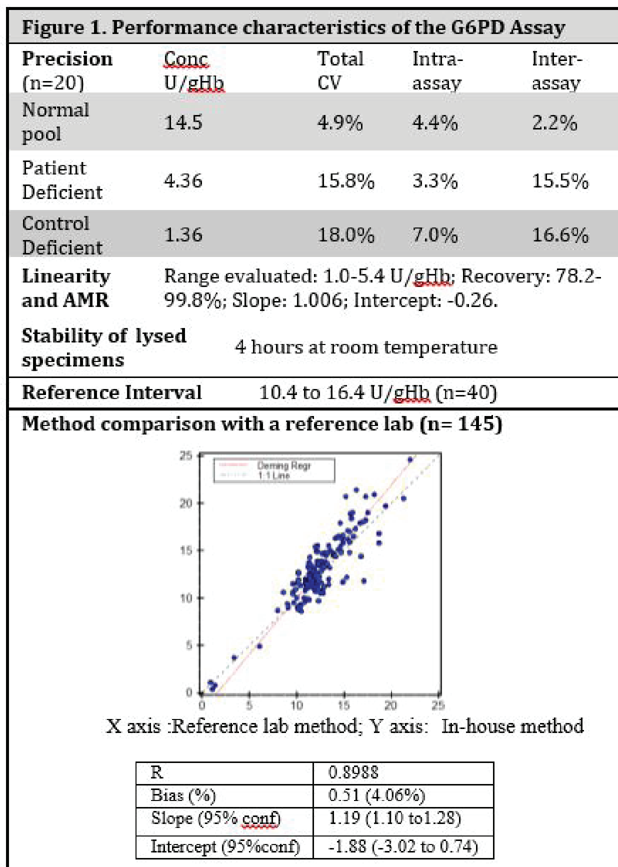


Figure 1. Performance characteristics of the G6PD Assay

Precision (n=20)	Conc U/gHb	Total CV	Intra-assay	Inter-assay
Normal pool	14.5	4.9%	4.4%	2.2%
Patient Deficient	4.36	15.8%	3.3%	15.5%
Control Deficient	1.36	18.0%	7.0%	16.6%
Linearity and AMR	Range evaluated: 1.0-5.4 U/gHb; Recovery: 78.2-99.8%; Slope: 1.006; Intercept: -0.26.			
Stability of lysed specimens	4 hours at room temperature			
Reference Interval	10.4 to 16.4 U/gHb (n=40)			

Method comparison with a reference lab (n= 145)

X axis :Reference lab method; Y axis: In-house method

R	0.8988
Bias (%)	0.51 (4.06%)
Slope (95% conf)	1.19 (1.10 to1.28)
Intercept (95%conf)	-1.88 (-3.02 to 0.74)

B-248

Using NASH-FibroTest to Assess Liver Fibrosis, Steatosis and Inflammation in Patients with Nonalcoholic Steatohepatitis (NASH): Between-Laboratory Comparability of Results and Correlation with Biopsy/Imaging Studies for a New, Non-Invasive, Blood Test

K. D. Galior, C. L. Rosemark, D. R. Block, N. A. Baumann. *Mayo Clinic, Rochester, MN*

Background: Nonalcoholic fatty liver disease (NAFLD) is a growing epidemic in the United States. Nonalcoholic steatohepatitis (NASH) is characterized by liver cell injury due to necroinflammatory processes in the presence of steatosis. Diagnosis and monitoring of NASH is an important aspect of patient management. The gold standard for diagnosing NASH is a liver biopsy followed by imaging studies (magnetic resonance elastography or ultrasound); however, liver biopsy is an invasive procedure with possibility of sampling errors while imaging techniques may be influenced by technical or patient-related factors. NASH-FibroTest (BioPredictive S.A., Paris, France) is a new, non-invasive, blood-based liver panel that uses 10 standard FDA-cleared serum biomarkers combined with the patient's age and gender to estimate the severity of NASH, steatosis and fibrosis in NAFLD. NASH-FibroTest includes 3 tests: NashTest 2, SteatoTest 2 and FibroTest to quantitatively assess liver inflammation, steatosis, and fibrosis, respectively. **Objective:** The objectives were to: i) compare NASH-FibroTest results from patient samples analyzed at Mayo Clinic (Rochester, MN) to results from the same samples analyzed at Pitié Salpêtrière Hospital (Paris, France) and ii) assess the clinical concordance between NASH-FibroTest results and findings from liver biopsy and/or imaging studies. **Methods**: Residual serum and plasma samples from 53 unique patients with diagnosis (N=44) or presumptive diagnosis of NASH (N=9) were obtained. NASH-FibroTest testing was performed according to BioPredictive technical recommendations in two clinical laboratories: Mayo Clinic, Rochester, MN and Pitié Salpêtrière Hospital, Paris, France. The NASH-FibroTest algorithm includes 10 biomarkers, age and gender. Plasma fasting glucose, serum apolipoprotein-A1, total bilirubin, gamma-glutamyl transferase, alanine aminotransferase, aspartate aminotransferase, total cholesterol and triglycerides (all measured on Roche Diagnostics, Inc. Cobas analyzers) and alpha-2-macroglobulin and haptoglobin (measured by nephelometry, Siemens Healthineers) were measured by each laboratory and numeric scores and stages/grades for FibroTest (F0-F4), NashTest 2 (N0-N3) and SteatoTest 2 (S0-S2S3) were calculated using Biopredictive's algorithm. The stages/grades obtained from each laboratory were compared. Electronic medical records were reviewed and clinical concordance between biopsy/imaging findings and NASH-FibroTest results was assessed in 10 patients for whom biopsy and/or imaging studies had been performed within 5 months prior to blood collection. **Results:** FibroTest stages obtained from the two laboratories were identical in 70% of patient samples (38/54) and agreed within 1 F-stage in 93% (50/54) of samples. NashTest 2 grades were identical in 85% (45/53) of samples and matched within 1 grade in 92% (49/53). One patient did not have N-stage calculated due to high fasting glucose. SteatoTest 2 grades were identical in 87% (47/54) of samples and agreed within 1 grade in 100% (54/54) of samples. For the clinical concordance assessment, 8 of 10 patients had NASH-FibroTest results that were concordant with findings from biopsy and/or imaging studies. In the two discordant cases, imaging studies detected diffuse hepatic steatosis and the SteatoTest 2 indicated <5% steatosis. **Conclusion:** Our study showed very good agreement between NASH-FibroTest results in samples analyzed at two different clinical laboratories. In addition, NASH-FibroTest results were found to be concordant with findings from imaging/biopsy studies in patients diagnosed with NASH.

B-249

Usefulness of C-Reactive Protein at Hospital Admission in Patients with Hip Fracture

Y. Kim[1], S. Kim[1], H. Kwon[1], H. Lee[1], Y. Park[1], J. Song[2], J. Lee[1]. *[1]Departement of Laboratory Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of, [2]Departement of Orthopedic Surgery, College of Medicine, The Catholic University of Korea, Seoul, Korea, Republic of*

Background: C-reactive protein (CRP) has been widely used to monitor the postoperative course in surgical trauma and to detect infection after orthopedic surgery. However, there have been very few studies on the usefulness of CRP measurement at hospital admission to detect infection in the patients with bone fracture. In this study, we examined whether the CRP measurement could be a useful marker in the detection of infection at hospital admission in the patients with hip fracture.

Methods: A total of 378 patients with hip fracture were included in this study. The patients were evaluated by age, gender, type of hip fracture, CRP level, bacterial culture, and time interval from injury to admission.

Results: Of the total 378 patients with hip fracture, 231 (61.1%) had intertrochanteric fracture, 129 (34.1%) had femur neck fracture, and 18 (4.8%) had subtrochanteric fracture. Of the 378 patients, 103 (27.2%) visited hospital within 3h after hip fracture, 90 (23.8%) visited from 3h to 6h, 60 (15.9%) visited from 6h to 9h, 27 (7.1%) visited from 9h to 12h, 49 (13.0%) visited from 12h to 24h, 27 (7.1%) visited at 2 days, 9 (2.4%) visited at 3 days, 4 (1.1%) visited at 4 days, 4 (1.1%) visited at 5 days, 4 (1.1%) visited at 6 days, and 1 (0.3%) visited at 7 days. CRP level in the patients visiting within 3h after hip fracture was the lowest concentration (mean of 1.06mg/dL) and the patients visiting at 2 days showed the highest concentration (mean of 6.27mg/dL). Of the 378 patients, 30 (7.9%) had infection at hospital admission: urinary tract infection (UTI) in 26 patients; bacteremia in 3 patients; pneumonia in 1 patient. CRP levels in the patients with infection and without infection were mean of 4.46mg/dL and 2.35mg/dL, respectively, which showed no statistical significantly difference between two groups.

Conclusions: Our data showed that the CRP measurement appears not to be useful in the detection of infection at hospital admission in the patients with hip fracture.

B-250

Potential Novel Biochemical Markers for NAFLD

G. Ajabnoor. *King Abdulaziz Unversity, Jeddah, Saudi Arabia*

Introduction: Non-alcoholic fatty liver disease (NAFLD) is a chronic condition progressing to liver cirrhosis. The standard diagnosis used to be based on biopsy, or ultrasound with some serious drawbacks. Non-invasive methods for the diagnosis are strongly needed. The aim of the present study is to study the relationship between plasma levels of FGF21, and plasma lipids, circulating levels of FFA and measures of insulin sensitivity (HOMA- IR, and QUICK), in diabetic individual with and without NAFL to help clarify the physiological roles of FGF21 in the development of NAFL disease.

Subjects and Methods: A total of 94 patients of type 2 Diabetic (T2DM) patients with NAFLD (n=50) & without (n=44), were recruited from clinics at king Abdul-

Aziz University Hospital-Jeddah in case- control design. All patients were screened for NAFLD by using abdominal ultrasound. They were then interviewed to fill a questionnaire for health indices that included medical history of chronic diseases. Height, weight, Waist, hip, and neck circumference (WC, HC, NC), and blood pressure were measured. Biochemical parameters were carried on fasting blood samples.

Results Almost all (98%) of NAFLD patients were overweight or obese, with significantly higher mean weight, WC, HC and NC. Moreover, a significantly higher percentage of above normal SBP & DBP were also noted. Means of liver enzymes, hs-CRP, triglycerides, total and LDL- cholesterol were significantly higher in NAFLD group (p value < 0.05 in all). There were also significant differences in the mean duration of being diabetic (P=0.003). Means of FGF-21, FFA, insulin resistance indices (HOMA-IR, QUICK, revised QUICK) were all higher in the NAFLD group. However, there was overlap in ranges of all variables between the two groups.

Conclusion: Collectively these findings suggested that obesity, and in particular abdominally obesity and hypertensive patients with highly increased levels of triglycerides, AST, GGT, and FGF 21 can be strongly suspected to have NAFLD. Therefore, it could be proposed that a combination of these parameters could be used to diagnose the disease.

B-251

Development of Monoclonal Antibodies to Pancreastatin, a Biomarker for Neuroendocrine Dysfunction

H. Stott, P. Ratcliffe, E. Mathers, J. W. Curry, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories, Crumlin, United Kingdom*

Background: Pancreastatin (PST) is a regulatory peptide, produced by proteolytic cleavage of chromogranin A (CgA). PST is involved in glucose insulin metabolism and exerts potentially multiple dysglycemic actions by inhibiting glucose-stimulated insulin secretion, inhibiting glucose uptake, inhibiting insulin-stimulated glycogen synthesis in hepatocytes, presenting lipolytic action in adipocytes. Increased PST levels have been reported in type 2 diabetes mellitus, gestational diabetes and hypertension, which could contribute to the development of insulin resistance.The serum concentrations of PST and CgA are increased in neuroendocrine tumours (NETs). The levels of PST are less influenced by factors like medication. For example, unlike CgA, PST levels are not elevated by proton-pump inhibitor (PPI) therapy. This study aimed to develop monoclonal antibodies (mAbs) for the detection of PST with the potential to be used in the development of efficient immunoassay-based methodologies in clinical settings. **Methods:** Sheep were immunized with PST and peptide fragments of PST. Lymphocytes B were collected and fused with heteromyeloma cells. Supernatants from the resulting hybridomas were screened for the presence of specific antibody using enzyme-linked immunosorbent assay (ELISA) based assays. Positive hybridomas were cloned to produce stable monoclonal hybridomas. The antibodies were purified and evaluated by direct binding ELISA to determine their specificity for PST and cross-reactivity with human CgA, non-amidated C-terminal PST and polyglutamate repeating domains common to PST and CgA. Epitope binning assays were then conducted using biolayer interferometry.

Results: Preliminary direct ELISAs showed that the developed mAbs could be sub characterized into three groups. One group of PST specific antibodies presented minimal cross-creativity (mostly <1 % under conditions tested) with human CgA, nonaminated C-terminus of PST and poly-glutamate repeating domains (common to PST and CgA). The two other groups of mAbs developed could be used to detect CgA and PST as they presented minimal cross-reactivity (<1 % under conditions tested) to the poly-glutamate repeating domain but did respond (with varying degrees) to CgA and screeners for the C-terminus of PST. The epitope binning assays allowed the subcategorization of the developed antibodies into PST specific and CgA/PST generic panels. All antibodies selected as PST captures fell into the same bin during Octet screening and paired with randomly selected CgA/PST generic antibodies. **Conclusion:** The results indicate the development of mAbs specific to PST with minimal cross-reactivity with human CgA as well as mAbs that recognise both PST and CgA under ELISA conditions tested. The use of the specific PST antibodies as captures and the CgA/PST antibodies as detectors will aid in the development of immunoassay-based technologies for application in clinical investigation.

B-252

Hemoglobin A1c Determination: Comparative Evaluation of Three Different Methods

G. Liuzzi, R. Buonocore, M. Bussetti, M. L. De Angelis, A. R. Corno, W. P. Porreca, C. F. Perno, S. Pastori. *Grande Ospedale Metropolitano Niguarda, Milano, Italy*

Background: Hemoglobin A1C (HbA1c), which is the result of prolonged exposure of erythrocytes to high glucose concentrations, is measured to check for diabetes or prediabetes and to monitor glycemic control. Various methods, based on different technologies, are currently being used to measure plasmatic HbA1c. The aim of the study is the analytical comparison of three methods for HbA1c determination.

Methods: 203 patient samples (96 female, 107 male) were analysed with the following 3 mehods: Capillary Electrophoresis (CE) (Capillarys® Sebia, France), High Performance Liquid Chromatography (HPLC) (Tosoh®, Japan) and Boronate Affinity HPLC (BA-HPLC) (Menarini®, Italy). Statistical Analysis was performed with the MedCalc Statistical Software version 18.11.3 (MedCalc Software bvba, Ostend, Belgium). The Friedman test was used to evalutate the differences among the three instruments; the Wilcoxon signed rank test was used to investigate statistical differences between paired measnurements. Analytical concordance was evaluated by the Passing-Bablok regression and Bland-Alman difference analysis.

Results: Measuremets (mmol/mol) obtained with the three methods resulted statistically different (Friedman P<0.00001). CE vs. HPLC and BA-HPLC vs. HPLC resulted in not statistically significant differences (P=0.8222 and 0.0942, respectively), whereas CE vs. BA-HPLC showed a statistically significant difference (P= 0.0004) (Wilcoxon). Passing-Bablok regressions and Bland-Altman differences are reported in the table.

Comparison	Passing-Bablok regression	95%CI slope	95%CI intercept
HPLC vs. CE	Y=-1.909+1.045X	1.000/1.088	-3.824/0.000
BA-HPLC vs. CE	Y=-1.000+1.000X	1.000/1.000	-1.000/-1.000
BA-HPLC vs. HPLC	Y=-2.286+1.071X	1.000/1.125	-4.625/1.000
Comparison	**Bland-Altman**	**95%CI**	**Limits of agreement**
HPLC vs. CE	0.079	-0.390/0.548	-6.562/6.719
BA-HPLC vs. CE	-0.315	-0.715/0.084	-5.974/5.344
BA-HPLC vs. HPLC	-0.394	-0.983/0.195	-8.738/7.949

Neither a systematic nor a proportional difference was observed when HPLC vs. CE and BA-HPLC vs. HPLC were evaluated with the Passing-Bablok regression analysis; on the contrary, a systematic difference was highlited in the BA-HPLC vs. CE comparison. This difference may be due to interference from the presence of Hb variants and derivatives. Bland-Alman difference analysis showed the absence of any statistically significant bias in all comparisons.

Conclusion: The three systems investigated, based on different methodologies, showed differences depending on the statistical method. Further evaluation from a clinical perspective would help evaluate the overall analytical performances.

B-253

Development of an Assay for Measurement of Total Immunoglobulin E (IgE) on Beckman Coulter AU Chemistry Analyzers

J. Lamboy, K. Boatman, M. Browning, M. Matal, Z. Zhu, M. Necula. *Beckman Coulter, Brea, CA*

Background: IgE is a member of the immunoglobulin family of proteins that are produced by plasma cells in response to antigenic stimuli. IgE differs from the other immunoglobulins in certain structural aspects, its role in allergic diseases, and the extremely low normal levels of circulating IgE in comparison to other immunoglobulins. Elevated levels are commonly seen in cases of allergic diseases and other conditions. Measurement of serum total IgE aids in the diagnosis of IgE-mediated allergic disorders in conjunction with other clinical findings.

Methods: An assay to measure total IgE in human serum and plasma was developed for use on AU chemistry analyzers*. Anti-IgE antibody-coated particles bind to IgE in the serum/plasma patient sample resulting in the formation of insoluble aggregates, causing turbidity. The amount of particle aggregation is directly proportional to the concentration of IgE in the sample. The assay was standardized to the 3rd WHO Inter-

national Standard for human serum IgE. This study aimed to evaluate the performance of the Total IgE assay on AU chemistry analyzers.

Results*: In development studies, within-run imprecision was ≤3.8 IU/mL SD and ≤2.8% CV (COV = 71.4 IU/mL) and total imprecision was ≤3.6 IU/mL SD and ≤3.3% CV (COV = 93.3 IU/mL) in the sample concentration range 51.5 - 482.2 IU/mL (CLSI EP05 abbreviated study, 10 days). The assay was linear in the measuring range of 20 - 500 IU/mL. In sensitivity studies, the assay exhibited a limit of blank <10.0 IU/mL and limit of detection <15.0 IU/mL, with a limit of quantitation of 20.0 IU/mL with <35%CV. Methods comparison studies (CLSI EP09-A3, n = 64-68) against the existing Roche Cobas IgE assay yielded slopes ranging from 0.95 to 0.99 and intercepts ranging from 0.19 to 5.59 using weighted Deming regression analysis. Less than 6% bias was observed from bilirubin, intralipid and hemoglobin interference studies.

Conclusion: In development studies, the Total IgE assay on AU systems demonstrated acceptable performances for sensitivity, linearity, precision, interferences and methods comparison.

**The assay is pending submission and clearance by the United States Food and Drug Administration; not yet available for in vitro diagnostic use in the US. For investigational use only. The performance characteristics of this product have not been established.*

B-254

Performance Evaluation of Clinical Chemistry Assays on the Atellica CH 930 Analyzer

D. Waltrick, L. Rodrigues, L. B. Avallone, C. O. W. Sabino, C. M. M. Dias, E. A. Perobelli, G. A. Campana. *DASA, São Paulo, Brazil*

Background: The primary objective of this study was to demonstrate the analytical performance of clinical chemistry assays for use in the Atellica CH 930 Analyzer, Siemens Healthineers. Studies included imprecision evaluation, linearity, and method comparison. **Methods:** Amylase, Antistreptolysin O, Direct and Total Bilirubin, Calcium, Cholesterol, Cholinesterase, Alkaline Phosphatase, Phosphorus, Glucose, Lipase, Magnesium and Protein assays from Atellica CH utilize the same reagents and calibrators from the respective assays in ADVIA Chemistry. The imprecision was verified through the study of repeatability (% CVR) and run-to-run variation (% CVWL) according to EP15-A3 and method comparison according to EP09-A3. For the imprecision study, two or three concentrations were used; each level of QC materials was tested in one run per day with five replicates per run for five days, resulting in a total of 25 replicates per sample for each run. Method comparison studies were performed using at least 40 serum samples that covered the assay range. **Results:** The imprecision results agreed with the analytical quality specifications. The %CVR was from 0.310% to 2.632% and %CVWL were 0.367% to 4.506% among all assays tested of the Atellica CH Analyzer. The comparison results of Passing & Bablock methods (R^2) ranged from 0.9903 to 0.9999. It was observed that 100% of the differences are within the calculated TE for each difference (EDA minimum 95%) for all trials. The assays tested on Atellica CH Analyzer and ADVIA Chemistry demonstrated excellent agreement. **Conclusion**: All assays tested on the Atellica CH 930 Analyzer have demonstrated acceptable results of imprecision and method comparison with the ADVIA Chemistry 2400 assays. The imprecision results are consistent with the analytical quality specifications. The study demonstrated that there is no clinical impact on the trials tested in a possible change for the Atellica CH 930. **Siemens Healthineers supported the studies by providing systems, and reagents.*

B-255

Performance Evaluation of New Sentinel CH - Atellica Alliance Applications Total Bile Acids, CK-MB Liquid and Aldolase

A. Cugini[1], S. Brambilla[1], D. Landenna[1], N. Di Rocco[1], J. Thomas[2]. [1]*Sentinel CH SpA, Milan, Italy,* [2]*Siemens Healthineers, Newark, DE*

Background: The purpose of the investigation was to evaluate the performance of the Sentinel CH reagents Total Bile Acids (TBA), Creatine Kinase MB (CK-MB) and Aldolase (ALD) on Siemens Atellica CH Analyzer. TBA are a marker for liver function, CK-MB is used for the diagnosis and follow-up of acute myocardial infarction and some muscular diseases while ALD is of great clinical interest in primary diseases of skeletal muscle (Duchenne-like muscular dystrophy, dermatomyositis and polymyositis). TBA assay utilizes the enzymatic cycling colorimetric method in which bile acids are converted to their corresponding oxosteroids in the presence of NADH and thio-NAD^+. Thio-NAD^+ is reduced to thio-NADH and its rate of production is proportional to the amount of TBA present in the sample. CK-MB assay uses an antibody-based inhibition method of CK-MM: creatine kinase releases ATP from creatine phosphate. ATP is used to form glucose-6-phosphate that reacts with $NADP^+$ forming 6-phospho-gluconate and NADPH. The absorbance variation due to the transformation of $NADP^+$ in NADPH allows to calculate CK-MB residual activity after CK-MM inhibition. Aldolase converts fructose-1,6-diphosphate to glyceraldehyde-3-phosphate and dihydroxy-acetone-phosphate. This last one, together with NADH, is converted into glycerol-1-phosphate and NAD^+. The rate of the aldolase reaction is measured by the decrease in absorbance as a consequence of the conversion of NADH to NAD^+.

Methods: Performance evaluation included Limit of Blank (LoB), Limit of Detection (LoD), Limit of Quantitation (LoQ), intra-assay imprecision, inter-assay imprecision, on board reagent stability, lot calibration interval, linearity, method/instrument correlation and sample carry over following the current CLSI guidelines protocols. Data were evaluated using Microsoft Excel statistical tool Analyse-it.

Results:

	TBA	CK-MB	ALDOLASE
LoB *	0.1 µmol/L	2.3 U/L	0.7 U/L
LoD *	0.2 µmol/L	4.1 U/L	1.1 U/L
LoQ *	0.5 µmol/L	5.5 U/L	1.7 U/L
Intra-assay imprecision	CV% 0.8% – 2.3%	CV% 0.3% – 9.4%	CV% 2.1% – 4.0%
Inter-assay imprecision	CV% 1.2% – 3.2%	CV% 1.4% – 7.0%	CV% 2.9% – 4.8%
On board reagent stability (up to 30 days + 10%)	Bias% -2.9% – -7.8%	Bias% -7.4% – 10.0%	Bias% -7.2% – 4.6%
Lot calibration interval (up to 30 days + 10%)	Bias% -6.3% – 2.42%	Bias% -9.6% – 8.9%	Bias% -8.5% – 1.3%
Linearity *	Linear 3 -- 90 µmol/L Linear 90 - 200 µmol/L	Linear up to 1036.3 U/L	Linear up to 34.5 U/L
Method / Instrument comparison	Method: y = 1.088 x + 0.227 µmol/L r = 0.999 Instrument: y = 0.979 x + 0.015 µmol/L r = 0.999	Instrument: y = 1.000 x + 0.000 U/L r = 1.000	Instrument: y = 0.891 x + 0.479 U/L r = 0.988
Sample carry over	Bias% 2.34% (0.01% considering the gap between the two concentrations	Bias% -3.85% (-0.09% considering the gap between the two concentrations)	Bias% 1.68% (0.74% considering the gap between the two concentrations)

*: performed on two batch

Conclusions: All assays tested demonstrated acceptable results for all the parameters tested on the Atellica CH Analyzer.

B-256

Performance Evaluation of Specific Proteins Assays on the Atellica CH 930 Analyzer in Brazil

F. Brito, W. Pedrosa. *Instituto Hermes Pardini, Vespasiano, Brazil*

Background: Specific proteins are valuable markers that enable diagnosis and management of metabolic and immune system-related diseases. Studies at our institution were performed to assess the analytical performance of specific proteins (SP) listed in tables 1 and 2 for the Atellica® CH 930 Analyzer with respect to method comparison with Beckman Coulter AU5800® System and, for lipoprotein assay, the method comparison with BN™ II System. **Methods:** Precision verification was performed according to EP15-A3. method comparison by EP09-A3. Method comparison studies were performed on 36 to 101 serum samples whose concentrations covered each assay range. from low to high. The results were analyzed using statistical tools and specifications of analytical quality. **Results:** The comparison results of Spearman's methods (R^2) ranged from 0.950 to 0.999. Method comparison studies are summarized in tables below. **Conclusions:** All assays tested on the Atellica® CH 930 Analyzer demonstrated good precision and correlation to the current AU5800® System or BN™ II System assays. The precision results were consistent with manufacturer's claims. **Siemens Healthineers supported the study by providing systems and reagents.*

Table 1. Method Comparison Data

Assay	Units	Method Comparison		Assay	Units	Method Comparison	
		Distribution of Differences	Linear Regression			Distribution of Differences	Linear Regression
IgG	mg/dL	-8.6% to +9.5%	Y = (-53.793) + 1.052 X	**APO B**	mg/dL	-6.3% to +19.3%	Y = (-0.875) + 1.062 X
IgM	mg/dL	-15.1% to +4.5%	Y = (-2.981) + 1.018 X	**Lipoprotein**	mg/dL	-3.5% to +112.6%	Y = (-3.088) + 1.322 X
IgA	mg/dL	-3.1% to +18.5%	Y = (-2.103) + 1.025 X	**Haptoglobin**	mg/dL	-7.2% to +9.4%	Y = (- 3.000) + 1.000 X
AGP	mg/dL	-10.2% to +18.8%	Y = (-9.562) + 1.184 X	**ASO**	UI/ mL	-23.9% to +107.47%	Y = (-1.503) + 1.157 X
AAT	mg/dL	-18.1% to +3.0%	Y = (-12.172) + 1.069 X	**B2M**	mg/L	-27.5% to +29.0%	Y = (-0.264) + 1.087 X
C3	mg/dL	-7.2% to +4.4%	Y = (-5.631) + 1.023 X	**hs-CRP**	mg/L	-8.8% to +21.7%	Y = (-0.090) + 1.186 X
C4	mg/dL	-22.4% to -8.9%	Y = 0.127 + 0.840 X	**PCR**	mg/L	-16.7% to +18.4%	Y = (-0.100) + 1.000 X
APO A-1	mg/dL	-17.6% to +22.3%	Y = (-15.838) + 0.891 X	**ASO**	UI/ mL	-23.9% to +107.47%	Y = (-1.502) + 1.157 X

B-257

Performance Evaluation of Hepatic Function Biomarkers Assays on Atellica CH 930 and Atellica IM 1600 Analyzers

D. Waltrick, L. B. Avallone, L. Rodrigues, C. O. W. Sabino, C. M. M. Dias, E. A. Perobelli, G. A. Campana. *DASA, São Paulo, Brazil*

Background: Several liver diseases initially present nonspecific signs. Therefore, the dosage of unmodified substances such as ALT, AST, GGT, Bilirubin and Albumin is a tool for the evaluation and identification of functions in liver and biliary function. In order to analyze the performance of the main assays that measure this substances through a large laboratory located in Brazil, it was evaluated the analytical performance of Albumin, AST, GGT and ALT assays on Atellica CH and Carcinoembryonic Antigen (CEA) on Atellica IM Analyzer (Siemens Healthineers). The studies included the imprecision verification, linearity and method comparison. **Methods:** The imprecision was verified through the study of repeatability (% CVR) and run-to-run variation (%CVWL) according to EP15-A3 and the methods comparison according to EP09-A3, and the linearity study according to EP06-A. For the imprecision study, two or three concentrations were used; each level of QC materials was tested in one run per day, with five replicates per run, for five days, resulting in a total of 25 replicates per sample for each assay. Method comparison studies were performed using at least 40 serum samples that covered the assay range. The number of linearity material levels ranged up to seven, depending on the assay. Each assay was tested in triplicate per sample concentration. **Results:** The imprecision results are in agreement with the analytical quality specifications. The %CVR was 0.663% to 5.565% and %CVWL was 0.821% to 5.425% among all the Atellica CH and Atellica IM Analyzer assays. The Passing & Bablock methods comparison results ranged from (R^2) 0.9903 to 0.9999. Linearity results were obtained for all assays. The assays tested on the Atellica CH Analyzer and ADVIA Chemistry demonstrated good agreement, as well as the assays tested on the Atellica IM Analyzer and the ADVIA Centaur XPT. Imprecision and method comparison results are listed below.

Assay	Units	PRECISION			METHOD COMPARISON		
		Mean conc.	Repeatability %CV (SD)	Run-to-Run Variation %CV (SD)	Atellica CH Analyzer Assays vs. ADVIA Chemistry System Assays	Pearson Coeficient	n
CEA	ng/mL	2.192	3.543 (0.078)	5.425 (0.119)	y = 0.977*X + 0.184	R^2 = 0.9876	46
		14.27	2.089 (0.298)	3.509 (0.501)			
		35.6	1.056 (0.376)	2.208 (0.786)			
ALBUMIN	g/dL	4.016	5.565 (0.023)	1.699 (0.068)	Y = 0.979*X + 0.066	R^2 = 0.9941	54
		2.936	1.028 (0.030)	1.381 (0.041)			
AST (TGO)	U/L	40.32	1.607 (0.648)	3.025 (1.220)	Y = 0.965*X + 0.332	R^2= 0.9996	44
		223.12	0.364 (0.812)	0.838 (1.871)			
GGT	U/L	63.183	2.245 (1.419)	2.137 (1.350)	Y = 1.025*X + 0.625	R^2 = 0.9999	42
		164.177	1.186 (1.947)	1.474 (2.420)			
ALT (TGP)	U/L	29.2	1.997 (0.583)	1.973 (0.573)	Y = 1.060*X + 0.108	R^2 = 0.9996	42
		102.8	0.663 (0.645)	0.821 (0.645)			

B-258

Development of Liquid-Stable Calibration Verification Control Kits for Human Serum Proteins to Characterize Method Linearity and to Validate the Reportable Ranges

A. Sample, C. Happe, J. Pawlak, M. Sweatt, R. Ito. *LGC Maine Standards, Cumberland Foreside, ME*

Introduction: Serum proteins are tested for a variety of reasons, including immune status of patients, nutritional assessment, and as indicators of acute or chronic inflammation, anemia, and specific disease states. There are numerous assays cleared by the FDA as non-waived laboratory tests. In accordance with CLIA ’88, clinical laboratories are therefore required to verify the manufacturer’s assay performance characteristics, including accuracy, precision and reportable range (AMR).

LGC currently provides two serum protein panels for calibration verification purposes. These products are five-level, liquid-stable, human serum based control kits and include the following biomarkers: Albumin, Alpha-1-antitrypsin, Ceruloplasmin, C3, C4, CRP, Haptoglobin, IgA, IgG, IgM, Prealbumin, Rheumatoid Factor and Transferrin.

To further support the needs of clinical laboratories, LGC’s objective was to extend its offering of serum protein calibration verification kits by developing a third panel that included anti-streptolysin O (ASO), Alpha-1-acid glycoprotein (AAG), Beta-2 Microglobulin (B2M) and Immunoglobulin E (IgE).

Methods: VALIDATE® SP3 was formulated as a multi-constituent product in a human serum matrix according to CLSI EP06-A, with five equal-delta concentrations to cover the reportable ranges of the Roche cobas®.

For each level, samples were tested in triplicate on the Roche cobas® 6000 analyzer for each of the 4 analytes. Reported recoveries were evaluated for mean, SD and linearity using MSDRx®, LGC’s proprietary linearity software. Limits were applied as a percentage of the total allowable error (TE_a), specific for each analyte. Product stability was determined by a combination of stress and real-time stabilities.

Results: For VALIDATE® SP3, all levels were stable, recovering within ±10% of the recovered values on the date of manufacture in stress studies, for each biomarker: ASO, AAG, B2M and IgE. Linear regression analysis of theoretical versus mean recovered values demonstrated linear performance of each assay through the manufacturer’s reportable range and within the applied TE_a limits. Real-time stability studies are ongoing.

Conclusion: Performance and stress-stability data supports that VALIDATE® SP3, as a five-level, liquid-stable, ready-to-use, control kit, is fit-for-purpose as a calibration verification test kit. The formulation continues in development, pending real-time stability assessments.

B-259

An Evaluation of the Abbott ARCHITECT® i2000 Procalcitonin (PCT) Versus bioMérieux VIDAS®: Chemiluminescence versus Kinetic Florescence Methodologies

P. Mingola, M. Quinton, D. Atwi, M. Maldonado-Vital, K. E. Blick. *Un of OK Health Sci Ctr, Oklahoma City, OK*

Background: As procalcitonin (PCT) becomes more widely used as an acute phase marker for diagnosis and management of bacterial infection, the laboratory must have a totally automated, rapid efficient assay for PCT readily available on a 24/7 basis. In this study, we compared our current semiautomated Brahms PCT assay run on the bioMérieux Vidas platform to PCT results obtained on the more automated Abbott Architect i2000. **Methods:** Laboratory specimens from 27 patients were used to perform a linear regression correlation study on Architect versus Vidas. Six manufacture-provided control specimens of known levels were used to evaluate PCT precision on the Architect at designated low, medium, and high values. In addition, six commercial control samples with values ranging from 0.10 to 100.0 ng/mL were assayed in a recovery study to further establish Architect PCT accuracy and validate assay linearity. **Results:** Linearity was confirmed within the manufacturer's range of 0.10 to 100.0 ng/mL with excellent correlation (r^2=0.999) and acceptable linearity (Architect PCT = 0.948*x + 0.338 ng/mL; where x equals PCT target values). Correlation on samples (n=24) between methods showed excellent correlation (r^2=0.998) with a regression line as follows: Architect=0.957*Vidas - 0.529 ng/mL; with some negative bias beyond the clinical interval of 0.00 to 2.00 ng/mL being observed. Within-run precision studies were as follows: low control, n=20, CV=2.90% , and high control, CV=1.90%, n=20. Results on between-run precision studies were as follows: low control, n=20. CV=3.10%; high control, n=20, CV=3.60%. **Conclusion:** Based on our comparison study results, the Abbott Architect i2000 and Brahms PCT assays show excellent linear correlation. Precision on the Architect PCT method is excellent. We conclude therefore that the Architect method is an excellent option for rapid 24/7 analysis of PCT and, because of the lack of bias between the two methods, results can be utilized clinically with essential no significant changes in the Vidas cutoffs being required. Because of the continuous availability of the totally automated Architect, PCT results can be obtained with very little hands-on technologist time being required.

Wednesday, August 7, 2019

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

TDM and Toxicology

B-260

Plasma Concentrations of Antituberculosis Drugs in Relation to Hepatic Enzymes in Tuberculosis Patients:a Prospective Analysis

A. A. Mir[1], S. Sharma[2], A. K. Duseja[2]. *[1]ESIC Medical College & Hospital, Hyderabad, India, [2]Postgraduate Institute of Medical Education and Research, Chandigarh, India*

Background: Isoniazid (INH) and Rifampicin (RIF) are generally well tolerated by tuberculosis (TB) patients. Effectiveness of treatment is compromised by drug induced hepatotoxicity (DIH). The aim of this study was to evaluate the anti-TB drug levels and the impact of serum levels of drugs on hepatic enzymes (ALT/AST) during treatment of TB. **Methods:** Study subjects were confirmed cases of tuberculosis on anti-TB drugs. Blood samples were obtained 1 hour after ingestion of anti-TB drugs. Blood was collected after at least 7 days of starting treatment. For liver function tests (LFT) blood was collected in plain vials. For estimating drug levels EDTA tubes were used. After separation, plasma was immediately stored at -20°C till analysis. At the time of HPLC (High Performance Liquid Chromatography) analysis, protein-free filtrates were made by using 10% trichloroacetic acid in case of INH and methanol for RIF. Finally, the filtrates were subjected to HPLC system. Reference ranges are 3-5 µg/ml (INH) and 8-24 µg/ml (RIF). **Results:** Comparative study of all groups was done on the basis of LFT and plasma ant-TB drug levels (Table1). Drug levels are significantly higher in patients having serum ALT/AST>25 IU/l. A significant correlation is seen between duration of anti-TB treatment and plasma anti-TB drug levels. **Conclusion:** As plasma anti-TB drug levels are significantly higher in patients with higher liver transaminases, their association to the DIH cannot be totally excluded. This finding might represent indirect evidence that suggests that high levels of plasma INH and RIF play a role in DIH. However, due to small number of study subjects and wide variability in the plasma levels of anti-TB drugs, we are not able to make recommendations that high level of serum ALT/AST can be used as a predictor for high plasma levels of anti TB drugs or vice versa, later during the course of treatment.

Table 1: Comparative laboratory outcomes and plasma concentrations of anti-TB drugs during the course of tuberculosis treatment:

Parameter	Group IA AST<25, IU/l	Group IB AST>25, IU/l	P value	Group IIA ALT<25, IU/l	Group IIB ALT>25, IU/dl	P value
ALT, IU/l (mean ± SD)	15.24±6.7	31.84±14.67	<0.0001	15.3±5.3	39.02±12.18	<0.0001
AST, IU/l (mean ± SD)	18.7±5	40.85±14.2	<0.001	23.9±10	42.3±15.8	<0.0001
ALP, IU/l (mean ± SD)	110.6±52.7	142.4±60.5	0.0262	118.6±59.7	142.3±54.7	0.07
LDH, IU/l (mean ± SD)	332.6±84	341±168	0.86	346.9±134.6	320±113	0.81
Total Bilirubin mg/dl (mean ± SD)	0.31±0.13	0.67±0.55	0.002	0.4±0.3	0.66±0.59	0.07
Direct Bilirubin mg/dl (mean ± SD)	0.117±0.05	0.23±0.26	0.034	0.13±0.08	0.25±0.3	0.19
Total Protein g/dl (mean ± SD)	7.85±0.48	7.86±0.69	0.77	7.94±0.54	7.7±0.68	0.11
Albumin g/dl (mean ± SD)	4.7±0.6	4.9±0.7	0.45	4.8±0.67	4.9±0.76	0.7
Duration of treatment, Days (mean ± SD)	63.3±54	94±86	0.0531	74.9±61	96.2±58	0.18
INH, µg/ml (mean ± SD)/ Median IQ Range	4.33±3.01 4.1 1.3-6.5	7.3±4.6 6.9 3.9-9.2	0.03	3.5±2.3 3.3 1.1-5.4	5.57±2.4 5.3 3.3-5.3	0.032
RIF, µg/ml (mean ± SD)/ Median IQ Range	1.9±3.5 0.83 0.057-1.95	3.2±3.3 2.6 0.97-4.5	0.01	1.6±1.7 1.1 3.3-5.3	3.2±2.2 2.9 1.17-4.5	0.01

P value<0.05 is significant

B-261

Exposure to Embalming Fluid and Changes in Biomarkers of Oxidative Stress, Reproductive Hormones and Renal Functions in Embalmers in Calabar, Nigeria

A. C. Nsonwu-Anyanwu, S. J. Offor, E. R. Ekong, C. A. O. Usoro. *University of Calabar, Calabar, Nigeria*

Background: Occupational exposure to formaldehyde in embalming fluid has been linked to chronic health conditions; lipid peroxidations, depletion of antioxidants and oxidative stress have been implicated as pathologic mechanisms. The biomarkers of oxidative stress, reproductive hormones and renal functions of embalmers in relation to duration of exposure to embalming fluid were determined in this study.

Methods: Sixty men aged 18-60 years comprising 30 embalmers and 30 non-embalmers were recruited into this case control study. Biomarkers of oxidative stress (malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), total antioxidant capacity (TAC), total plasma peroxides (TPP)) and renal functions (urea and creatinine) were estimated by colorimetry, reproductive hormones (leutinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone) by enzyme linked immunosorbent assay method and urine formic acid (FA-U) by gas chromatography. Data were analysed using analysis of variance and Pearson's correlation at p<0.05.

Results: Embalmers had higher urine formic acid, lipid peroxides and lower glutathione, nitric oxide, total antioxidant capacity and testosterone compared to non-embalmers (p<0.05). Increasing duration of exposure to embalming fluid is associated with increased urine formic acid and decreased FSH levels. MDA correlated negatively with TAC (r =-0.396, p=0.030) and positively with OSI (r=0.519, p=0.003) only in embalmers.

Conclusion: Chronic exposure to formaldehyde in embalming fluid is associated with increased formic acid excretion, lipid peroxidation and depletion of antioxidants, testosterone and FSH which may result in perturbations in male reproductive functions in embalmers studied.

B-262

Development and Validation of a Bioanalytical Method for Certolizumab Pegol (Cimzia) Drug Using Surface Plasmon Resonance

M. Kelemen, K. Chun, B. Holmquist. *Esoterix/LabCorp, Calabasas, CA*

Background: Surface Plasmon Resonance is a powerful technology for characterization of ligand binding interactions between biological molecules. The biosensor detects changes in optical resonance when an analyte binds to a molecule that has been immobilized to a gold surface. Few descriptions of the use of SPR in the clinical laboratory for quantitative analysis have been reported.

Objective: Certolizumab Pegol (Cimzia®) is a biologic drug that is a PEGylated Fab' fragment of a monoclonal antibody specific to tumor necrosis factor alpha (TNF-α). Certolizumab Pegol and other biologics are monoclonal antibody therapeutic proteins (MATs) used to treat a variety of diseases. Therapeutic drug monitoring (TDM) of drug and anti-drug antibodies (ADA) is often used to evaluate individual responses to MATs and guide personalized treatment regimens. In this study a ligand binding assay for the biologic drug Certolizumab Pegol using Surface Plasmon Resonance (SPR) technology was developed.

Methods: Anti TNF-α Antibody is covalently immobilized onto a SPR biosensor by amine coupling. TNF-α is then flowed over the biosensor and acts as a capture molecule for the Certolizumab Pegol in standards, controls, and patient samples. Next, standards, controls, and unknowns are diluted in phosphate buffer before being flowed over the biosensor. Finally, an antibody specific to Certolizumab Pegol is flowed over the biosensor to add specificity and enhance the final signal obtained in each sensorgram. The final enhanced signal is measured, and the Certolizumab Pegol concentration in each unknown sample and control is interpolated from a standard curve generated with each assay.

Results: The analytical sensitivity was 1 ug/mL with an analytical measurement range up to 90 ug/mL (up to 900 ug/mL on dilution). Inter-assay precision ranged from 4.0% to 8.0%. Inter-assay accuracy, as measured by spike recovery, ranged from 96.5% to 113.9%. The method was verified to be specific for Certolizumab Pegol by testing alternative biologics that are targeted to TNF-α including Adalimumab, Golimumab, Infliximab and Enbrel. No interference was observed in the presence of hemolysis, icteric or lipemia. No difference in result was observed when using collection tubes containing EDTA, Heparin or gel barriers. Specimen stability was interrogated at ambient, refrigerated and frozen conditions. Samples from patients prescribed Certolizumab Pegol were tested as part of the validation.

Conclusion: A method for quantitation of Certolizumab Pegol has been validated for clinical use using Surface Plasmon Resonance detection.

B-263

Vancomycin-Induced Acute Kidney Injury in Hong Kong

Q. Xu Zhen. *Peking Union Medical College Hospital, Beijing, China*

Background: To study the incidence of vancomycin-induced nephrotoxicity (VIN) in Hong Kong and identify the characteristics of susceptible patients and the most likely risk factors.

Method: This study was conducted using the Hong Kong Hospital Authority Clinical Data Analysis and Reporting System (CDARS). All the data of patients with vancomycin prescription and measurement from 2012 to 2016 in Hong Kong were retrieved from CDARS. Acute kidney injury (AKI) was defined using KIDIGO criteria. Patients without creatinine measurements, steady-state trough vancomycin level or who had vancomycin treatment <3 days were excluded. Results were analyzed using SPSS version 22.0. Logistic regression was used to identify the predictors for VIN.

Results: 1450 patients were identified as VIN from 12758 complete cases in Hong Kong from 2012 to 2016. The incidence was respectively 10.6%, 10.9%, 11.3%, 12.2%, 11.2% from 2012 to 2016. The baseline creatinine, trough vancomycin level and death in hospital were significantly higher in VIN group. Higher trough concentration of vancomycin was associated with a higher incidence of VIN (P<0.001). In logistic regression analysis, patients with higher baseline creatinine, chronic diseases and multiple drug treatment including diuretics, piperacillin-tazobactam, meropenem, ACEI, and ARB were associated with a higher risk of developing VIN.

Conclusion: The incidence of VIN in Hong Kong is low but shows no decline. Patients with higher baseline creatinine, multi-organ diseases and multiple drugs should have their vancomycin level monitored to decrease the risk of VIN.

B-264

Comparative Cannabinoid Cross-Reactivity in THC Immunoassays

G. M. Kroner[1], K. Doyle[2], K. L. Johnson-Davis[3], G. A. McMillin[3]. *[1]University of Utah Health Sciences Center, Department of Pathology, Salt Lake City, UT, [2]Intermountain Healthcare, Salt Lake City, UT, [3]University of Utah Health Sciences Center, Department of Pathology; ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT*

Background: Cross-reactivity is a common concern with immunoassays that are used to detect drug use. The active component of marijuana, tetrahydrocannabinol (THC), shares structural similarities with other cannabinoids. The increasing use of cannabinoid products makes it important for labs to be aware of potential cross-reactivity with immunoassays designed to detect THC and related metabolites. The objective of this study was to evaluate the extent of cross-reactivity of two THC immunoassays with four compounds: cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), and cannabigerol (CBG). **Methods/Results:** We first tested for cross-reactivity using three different urine pools spiked with 1000 ng/mL of each compound. Pools were prepared by mixing at least three deidentified patient samples that were negative for THC via the EMIT II Plus immunoassay. This revealed that one immunoassay, the Microgenics MultiGent Cannabinoids assay run on an Abbott Architect instrument, had no detectable cross-reactivity with any of the compounds, and that the other immunoassay, the Beckman Coulter Emit II Plus Cannabinoid assay, only showed notable cross-reactivity with CBN. CBN, an aromatized derivative of THC, is thought to exist in small concentrations in cannabis plants, but is mainly formed during aging and storage of cannabis products; it is also marketed as a sleep aid. We tested a range of spiked CBN concentrations to determine what concentration of CBN was required to trigger a positive immunoassay result. The immunoassay is qualitative: a response greater than the established cut-off value indicates that the sample contains more than 20 ng/mL THC metabolite. For CBN, we identified that a spike of 100 ng/mL or more produced a positive result. Finally, we established that CBN has an additive effect with THC. We spiked 21 individual samples that were weakly positive for THC via a mass-spectrometry method (between 5 and 10 ng/mL), but negative by the EMIT II Plus immunoassay, with a concentration of CBN not itself sufficient to yield a positive immunoassay response (50 ng/mL). While the response for all samples did increase and 13 of the 21 became positive by immunoassay following the addition of the CBN, we noted that not all samples responded to the spiked CBN to the same extent. **Conclusions:** Overall, this work illustrates the variability between immunoassay manufacturers and demonstrates that some immunoassays do have cross-reactivity with other cannabinoids. It is important to note that the purity of many cannabinoid products is not regulated, so products may also contain varying amounts of THC, which will complicate the interpretation of results. Regardless, it is important for manufacturers and labs to assess the cross-reactivity of assays carefully since false-positive drug screens can cause serious consequences for patients.

B-265

Performance of the Emit II Plus Oxycodone Assay on the Dimension Vista Intelligent Lab Systems

S. Taiba, G. E. Siefring Jr., B. Israel, N. Morjana. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Objective: Oxycodone, a semisynthetic opioid, is used for treatment of moderate to severe pain but has a high potential for abuse and addiction. A new protocol for measurement of oxycodone has been developed on the Dimension Vista® Intelligent Lab Systems. The Emit® II Plus Oxycodone assay has two cutoffs: 100 and 300 ng/mL. The assay consists of liquid, ready-to-use reagents that provide qualitative and semiquantitative results.

Methods: Precision was evaluated using the cutoff and ±25% controls according to CLSI EP5-A2. Recovery was assessed by spiking oxycodone into human urine at levels that span the assay range. Calibration stability was assessed by testing the cutoff and ±25% controls over a 31-day period. Specimens (100) for each cutoff were analyzed and the results compared to those from the Viva-E® Drug Testing System. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences with each cutoff was assessed by spiking the interferents into human urine in the presence of oxycodone at levels of ±25% of the cutoff.

Results: Qualitative repeatability CVs (rate) for the cutoff and ±25% controls ranged from 0.74 to 1.10%; within-lab CVs ranged from 1.24 to 1.50%. Semiquantitative repeatability (ng/mL) CVs ranged from 3.22 to 5.06%; within-lab CVs ranged from 4.91 to 5.68%. The overlap rate between the ±25% oxycodone controls and the cutoffs was less than 5%. Semiquantitatively, the assay quantified oxycodone-spiked samples

within ±20% of nominal values. At the cutoff levels, the percent agreement of specimens between the Dimension Vista 500 and Viva-E systems was greater than 95%. At 100 ng/mL cutoff, oxycodone reagents detect oxymorphone in urine at greater than 80%, with minimal cross-reactivity with structurally related drugs (0.01%). Potential interfering substances resulted in no false responses for the spiked ±25% controls relative to the cutoffs. A minimum of 14 days of calibration stability was demonstrated.

Conclusion: The new Emit II Plus Oxycodone assay on the Dimension Vista systems is a suitable screening method for urine specimens in both qualitative and semiquantitative analyses.

HOOD05162002983072

B-266

Performance Evaluation of the Atellica CH NAPA, PROC, and LIDO Assays

J. T. Snyder, J. Parker, J. Cheek, K. Kolewe, S. Janas, Z. Boyles, C. Theos. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH N-Acetyl Procainamide (NAPA), Procainamide (PROC), and Lidocaine (LIDO) Assays on the Atellica® CH Analyzer. Procainamide is an antiarrhythmic medication that, upon being metabolized, produces the active metabolite N-acetyl procainamide. NAPA and PROC assays are both useful in the monitoring of procainamide therapy. Lidocaine is also an antiarrhythmic agent and a local anaesthetic. The LIDO assay is useful in therapeutic drug monitoring and the diagnosis of lidocaine overdose. NAPA, PROC, and LIDO are homogeneous particle-enhanced turbidimetric inhibition immunoassays (PETINIAs). Analyte in the patient sample competes with an analyte-labeled particle for antibody-binding sites. The rate of aggregation is inversely proportional to the concentration of analyte in the sample.

Methods: Performance testing included precision and method comparison. Assay precision was evaluated using CLSI guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison studies were conducted according to CLSI EP09-A3, with patient sample results compared with results from the respective Dimension® EXL™ with LM Integrated Chemistry System assays.

Results: For NAPA, within-lab precision ranged from 1.1 to 4.5% CV for serum and plasma samples. For PROC, within-lab precision ranged from 1.0 to 2.3% CV for serum and plasma samples. For LIDO, within-lab precision ranged from 1.3 to 3.1% CV for serum and plasma samples. The NAPA method comparison study yielded a regression equation of $y = 1.04x - 0.1$ µg/mL, with r of 0.997. The PROC method comparison study yielded a regression equation of $y = 1.01x + 0.1$ µg/mL, with r of 0.998. The LIDO method comparison study yielded a regression equation of $y = 0.98x + 0.1$ µg/mL, with r of 0.998. These assays were compared with the respective Dimension EXL with LM assays.

Conclusion: The Atellica CH NAPA, PROC, and LIDO Assays tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement compared to the respective Dimension EXL with LM assays.

B-267

Stability of Amphetamines in Driedd Blood Spots Using GC Method

H. Alkhalaf[1], K. Mohammed[2], M. Elramady[2]. *[1]Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [2]Naif Arab University for Security Science, Riyadh, Saudi Arabia*

Background: Recently, analysis of dried blood spots (DBS) is an increasingly accepted method in therapeutic drug monitoring and forensic toxicology. Consequently, stability studies of validated Gas Chromatography Mass Spectrometry (GC-MS) method for amphetamine, methamphetamine (MA), methylendioxyamphetamine (MDA), methylendioxymethaphetamine (MDMA), and methylendioxyethylamphetamine (MDEA) in DBS are required. This study aimed at evaluating a (GC-MS) method to determine amphetamine and its derivatives in dried blood spots on filter paper and their stability during storage for 5 months at three different temperature degrees (room temperature °C, 4 °C, and -20°C).

Methodology: Twenty-one samples were analyzed for Amphetamine and its Derivatives. Sample preparation was included liquid-liquid extraction with ethyl acetate in the presence of sodium hydroxide as alkaline medium. Methamphetamine-D5 (MA-D5) and methylendioxyamphetamine- D5 (MDA-D5) were used as internal standards (IS). The sensitivity was evaluated by determining limits of detection (LOD) and quantification (LOQ). The precision was expressed as a Relative Standard Deviation % (% RSD) and the accuracy was expressed as % Bias for each analyte. The Linearity was estimated for all amphetamines. For quantification of each analyte, the principle ions *m/z* 240, 254, 375, 254, 268 were used as quantifier ions for Amphetamine, MA, MDA, MDMA, and MDEA, respectively.

Results and Discussion: The calibration curves were linear ($r = 0.98$) in the concentration range 50-2000 ng/mL for all analytes. The LODS based on signal-to-ratio (S/N) ≥ 3 were 25 ng/ml for amphetamine and its derivatives. The LOQS based on S/N ≥10 were 50 ng/ml for all analytes. The % RSD mean for all analytes which was within acceptable range (3.71-13.89). The % Bias ranged between (- 4.41%) and 8.90 % for amphetamine and its derivatives which was within the acceptable Total Error Allowable (15%). All analytes were found to be more stable at 4 °C and -20 °C. The % concentration changes when stored up to 132 days was ≤ 14 % . All analytes stored at room temperature were stable only for 28 days.

Conclusions: Overall performance of Amphetamine and its derivatives on GC-MS instrument was acceptable for clinical and forensic laboratories. Keeping in mind that the best storing condition for the samples is -20 °C.

B-268

A Method Comparison Study of the ARK™ Methotrexate Immunoassay on Abbott Architect Analyzers versus LC-MS/MS

L. M. Moore, L. V. Ramanathan, J. L. Thackray, E. K. Slotkin, D. Carlow. *MSKCC, NEW YORK, NY*

Background: Methotrexate (MTX) is an antimetabolite drug used for the treatment of various malignancies. MTX has a very narrow therapeutic window and may cause severe life-threatening nephrotoxicity. This necessitates continuous therapeutic drug monitoring (TDM). The most widely used Abbott TDx® MTX assay was recently discontinued. Thus, an alternative methodology for MTX measurement is urgently needed. Current methods available include: the FDA-approved ARK™ MTX immunoassay and liquid chromatography mass spectrometry (LC-MS/MS). The use of LC-MS/MS is limited, as it is not available at all institutions, whereas immunoassays represent a more feasible and convenient option for most laboratories. **Objectives:** The main goal of this study was to compare the analytical performance of the ARK™ MTX immunoassay on two C-series Abbott Architect automated analyzers versus LC-MS/MS. We focused specifically on the lower measurement range of MTX (0.04-0.6 µmol/L) because this range is crucial in clinical decision-making in the pediatric patient population at our institution. **Methods:** MTX concentrations were determined using a homogeneous enzyme immunoassay (ARK Methotrexate, ARK Diagnostics Inc) performed on the Abbott Architect c8000 and c16000 automated clinical chemistry analyzers. Samples were analyzed according to the manufacturer's protocol. The measurement range of the ARK™ MTX assay, as indicated by the manufacturer, was 0.04-1.20 µmol/L. Measurement of MTX by LC-MS/MS was performed using an established protocol with analytical performance characteristics published elsewhere [1]. For method comparison we obtained IRB approval and analyzed sixty residual human patient samples by both methods. Linear regression analysis and Bland-Altman difference plots were determined using Analyse-it (Analyse-it Software, Ltd, UK). Inter-day assay imprecision of both methods was assessed by measuring MTX at (low) 0.08 µmol/L, (medium) 0.20 µmol/L, and (high) 0.60 µmol/L concentrations over a period of twenty days. Additionally, we evaluated concentrations near the limit of quantification (0.04 µmol/L). **Results:** For method comparison linear regression analysis showed a strong correlation between the ARK™ MTX immunoassay and LC-MS/MS when the entire measurement range of MTX was examined (c8000; slope: 1.12, R^2: 0.94 and c16000; slope: 1.23, R^2: 0.93). However, the R^2-value decreased when we examined MTX at concentrations below 0.2 µmol/L; a clinically-relevant concentration near the cut-off used at our institution for clinical-decision making in pediatric patients. The R^2 for both C8000 and C16000 automated analyzers versus LC-MS/MS at concentrations <0.2 µmol/L decreased to 0.79. Additionally, Bland-Altman difference plots showed that the ARK™ MTX assay exhibited a positive bias compared to LC-MS/MS, with an average mean difference of 0.05 µmol/L. For the inter-day imprecision, the immunoassay on both automated analyzers were within acceptable limits with all CV% below 15% at low, medium, or high concentrations. **Conclusions:** Overall there is high agreement between the ARK™ MTX immunoassay and LC-MS/MS; however, agreement decreases at MTX concentrations below 0.2 µmol/L. Additionally, a clinically significant positive bias was observed, which showed that the immunoassay runs higher compared to LC-MS/MS. These results suggest that clinical-decision making may be impacted with use of ARK™ MTX immunoassay at lower concentrations.

References: Schofield RC, et al. J Chromatogr B Analyt Technol Biomed Life Sci .2015;1002:169-175

B-269

Clinical Application of a Simple and Reliable LC-MS/MS Method for Teicoplanin Drug Monitoring: 3 Year Experience at a Teaching University Hospital

J. Jung[1], K. Lee[2], J. Oh[1], R. Choi[3], H. Woo[1], H. Park[1], C. Kang[1], Y. Kim[1], S. Lee[1]. *[1]Samsung Medical Center, Seoul, Korea, Republic of, [2]Kangbuk Samsung Hospital, Seoul, Korea, Republic of, [3]Green Cross Laboratories, Gyeonggi-do, Korea, Republic of*

Background: Teicoplanin is a glycopeptide antibiotics used for treatment of Gram-positive bacterial infection instead of vancomycin. Teicoplanin consists of five major components (A2-1, A2-2, A2-3, A2-4, A2-5). To date, many studies have assessed only the main teicoplanin component (A2-2) or did not identify the individual components. We developed and validated an liquid-chromatography-tandem mass spectrometry (LC-MS/MS) method to measure serum concentrations of teicoplanin including the five major subcomponents. Also, we applied the assay method to therapeutic drug monitoring (TDM) of teicoplanin in our clinical patients. **Methods:** We established an LC-MS/MS method to measure serum concentration of teicoplanin using simple protein precipitation. Analyses were performed on a Xevo TQ-S tandem mass spectrometer equipped with an ACQUITY Ultra Performance LC. Chrlomatographic separations were performed using a Kinetex C18 100Å column. Quantification was performed using the ratio of the integrated peak area of teicoplanin (sum of A2-1, A2-2, A2-3, A2-4 and A2-5). The linearity, lower limit of quantitation a accuracy, precision, matrix effect, extraction recovery and carryover effects were evaluated. A total of 421 serum teicoplanin concentrations was measured in 223 patients from 2014 to 2017. We collected demographic and clinical information through review of medical records, and compared differences in drug concentration and evaluate therapeutic effectiveness between two age groups (<18 years, >=18 years). **Results:** Total chromatographic run time was 5.0 min. The calibration curve yielded a linear response from 2.0 to 100 µg/mL. The intra- and inter-assay imprecisions were CV <7.5% and <4.7%, respectively. The accuracy was less than ±10% bias. The lower limit of quantification was 0.2 µg/mL. The extraction recovery ranged from 88.8% to 96.6% for teicoplanin and from 9.76% to 109.8% for internal standard. In the clinical patient samples, the median relative proportions of each teicoplanin fraction were as follows: Teicoplanin A2-2/A2-3 38.1%, teicoplanin A2-4/A2-5 60.0%, teicoplanin A2-1 3.2%. Of 421 measurements, 87 (20.7%) were subtherapeutic (<10 µg/mL), and 4 (0.9%) were above the toxic threshold (>= 60 µg/mL). Patients were diagnosed with solid tumors (n=77), hematologic malignancies (n=51) or infectious disease (n=44). Adult patients accounted for 66% and pediatric patients accounted for 34%. Pediatric patients were administered a higher teicoplanin dose per unit body weight than adult patients but were found to have lower serum teicoplanin concentrations. Serum teicoplanin concentration was measured once in 140 patients (63%), and multiple measurements were completed for the others (83 patients, 37%). Intra-patient variability in teicoplanin concentration was found (CV 33%, range 2-94%). **Conclusion:** Our simple and rapid LC-MS/MS method was successfully applied in TDM of teicoplanin in clinical practice. This method is suitable for routine TDM in clinical care, as it requires only basic sample preparation and allows rapid quantitation of teicoplanin concentrations. Measurement of teicoplanin subcomponents is necessary and important to provide appropriate teicoplanin TDM.

B-270

Performance Comparison of the Enzyme Multiplied Immunoassay Technique (EMIT) and Particle-Enhanced Turbidimetric Inhibition Immunoassay (PETINIA) Valproic Acid (VPA) Assays

Y. Li, G. Wang. *Beijing Strong Biotechnologies. Inc, malden, MA*

Background: VPA is an antiepileptic drug whose curative effect and toxicity are related to its serum concentration. Beijing Strong Biotechnologies, Inc (BSBE) has developed a new EMIT VPA kit, in order to provide a convenient and cost effective option for clinical chemistry laboratories. **Methods:** The performance of the EMIT VPA assay was compared with the commercially available PETINIA reagent. 55 clinical samples were measured for method comparison based on the EP9-A2. 11-level VPA samples with the concentration range from 10 to 180 µg/ml were tested for linearity according to the EP6-A2. For recovery studies, high stock solutions of VPA from the United States Pharmacopeia (USP) reference material were prepared with dimethyl sulfoxide (DMSO), and then diluted 20 times with normal clinical serum for six different levels. Eight VPA analogs and metabolites were tested for cross reactivity. Other performances including precision, stability and interference were also determined. **Results:** The EMIT assay has a good correlation with the PETINIA reagent: EMIT = 1.0162 × PETINIA + 0.5699, $R^2 = 0.9945$. The linear range of the EMIT assay was 10-180 µg/ml, while that of the PETINIA assay was 10-150 µg/ml. All the other performance data were shown in Table 1. **Conclusion:** The EMIT VPA assay has better accuracy, broader linear range, and lower cross-reactivity, meanwhile the precision, stability and interference were comparative with the PETINIA kit.

Table 1A: Spike recovery data of the VPA and PETINIA kit. Each sample were tested 3 times and bias were calculated between target and mean recovery value.

sample	theoretical value (µg/ml)	EMIT		PETINIA	
		mean (µg/ml)	bias%	mean (µg/ml)	bias%
sample 1	25	23.1	-7.6%	22.6	-9.47%
sample 2	30	27.6	-7.9%	27.9	-7.11%
sample 3	50	50.1	0.1%	48.8	-2.47%
sample 4	75	77.0	2.6%	71.9	-4.13%
sample 5	100	103.3	3.3%	100.1	0.07%
sample 6	125	123.6	-1.1%	129.1	3.28%

Table 1B: Specificity data of the VPA and PETINIA kit. High stock solutions of 6 VPA analogs were diluted 10 times by the clinical serum pool with 100 µg/ml VPA.

analogue and metabolite	theoretical value (µg/ml)	EMIT		PETINIA	
		mean (µg/ml)	bias%	mean (µg/ml)	bias%
2-N-propyl-5-hydroxy-pentanoic acid	25	105.8	1.68%	103.8	6.46%
	50	110.7	6.44%	108.7	11.49%
2-N-propyl-3-hydroxy-pentanoic acid	100	107.6	3.46%	101.6	4.15%
2-N-propyl-4-hydroxy-pentanoic acid	100	106.9	2.74%	104.4	7.08%
2-N-propyl-3-oxo-pentanoic acid	100	99.3	-4.52%	94.7	-2.87%
2-propyl-2-pentanoic acid	20	99.9	-3.99%	98.4	0.92%
2-propylglutaric acid	200	121.7	16.97%	122.2	25.28%
2-N-propyl-4-pentanoic acid	50	115.1	10.63%	113.5	16.36%
	100	134.2	29.04%	130.3	33.64%
2-N-ethyl-2-phenylmalonamide	100	100.4	-3.51%	97.1	-0.46%

Table 1C: Precision data of the VPA and PETINIA kit. 4 controls were tested in replicate in two runs (interval for 2 hours at least) per day for a minimum of 20 days.

Sample	EMIT			PETINIA		
	mean (µg/ml)	Within Run CV	Total Run CV	mean (µg/ml)	Within Run CV	Total Run CV
Control 1	27.20	1.82%	3.71%	27.50	3.64%	4.55%
Control 2	119.80	2.15%	4.19%	121.10	0.94%	1.67%
Control 3	84.20	1.61%	2.80%	80.40	0.74%	1.74%

Table 1D: Interference studies of VPA and PETINIA kit. High stock solutions of interfering compounds were diluted 20 times by the clinical serum pool with 80 µg/ml VPA.

Interference Substence	theoretical value (mg/dl)	EMIT		PETINIA	
		mean (µg/ml)	bias%	mean (µg/ml)	bias%
Bilirubin unconjugated	20	78.1	-1.6%	76.1	0.6%
	30	77.3	-0.9%	75.1	-0.3%
Bilirubin conjugated	20	79.6	-2.3%	76.9	2.4%
	30	77.5	-1.7%	76.3	1.1%
Hemoglobin	800	81.3	1.7%	77.2	-0.3%
	1000	83.8	4.9%	76.9	-0.6%
Intralipid	1000	77.3	-3.7%	74.9	0.8%
	2000	74.5	-1.8%	68.3	-5.5%

B-271

Performance Evaluation of the Atellica CH OXY Assay

J. T. Snyder, J. Parker, J. Cheek, K. Kolewe, S. Janas, K. Hay, C. Theos. *Siemens Healthcare Diagnostics Inc, Newark, DE*

Background: The purpose of the investigation was to evaluate the analytical performance of the Atellica® CH OXY Assay on the Atellica® CH Analyzer. Oxycodone (OXY) is an opioid analgesic prescribed as a pain reliever and, because it structurally resembles and has similar effects as codeine and morphine, has the potential to be abused. The Atellica CH OXY Assay is intended for qualitative and semiquantitative determination of oxycodone in human urine. The assay provides only a preliminary analytical test result. A more specific alternative chemical method such as liquid chromatography/mass spectroscopy (LC/MS) must be used to obtain a confirmed analytical test result. The Atellica CH OXY Assay uses a homogeneous enzyme immunoassay technique, where competition occurs between the drug in the specimen and drug labeled with recombinant glucose-6-phosphate-dehydrogenase (rG6PDH) for antibody-binding sites. Enzyme activity decreases upon binding to the antibody. Active enzyme converts nicotinamide adenine dinucleotide (NAD) to NADH in the presence of glucose-6-phosphate (G6P), resulting in a change in absorbance that is measured spectrophotometrically. **Methods:** Performance testing included precision and method comparison at the 100 ng/mL and 300 ng/mL cutoff levels. Assay precision was evaluated using CLSI guideline EP05-A3. Each sample was assayed in duplicate twice a day for 20 days. Method comparison was tested on the Atellica CH Analyzer, with results compared to LC/MS.

Results: Within-lab precision was conducted over 20 days with both qualitative and semiquantitative output using 9 samples, which included 4 below the cutoff, 4 above the cutoff, and 1 at the cutoff. For the 100 ng/mL cutoff assay, all samples below the cutoff yielded negative results, and all samples above the cutoff yielded positive results. A sample at the cutoff yielded 57 negative and 23 positive results. For the 300 ng/mL cutoff assay, all samples below the cutoff yielded negative results, and all samples above the cutoff yielded positive results. A sample at the cutoff yielded 53 negative and 27 positive results. The method comparison studies included four sample

categories: low negative, near-cutoff negative, near-cutoff positive, and high positive. For the 100 ng/mL cutoff, agreement among positives was 51/51 (100%), and agreement among negatives was 41/49 (84%), both qualitatively and semi-quantitatively. For the 300 ng/mL cutoff, agreement among positives was 37/37 (100%), and agreement among negatives was 56/63 (89%), both qualitatively and semi-quantitatively. **Conclusion:** The Atellica CH OXY Assay tested on the Atellica CH Analyzer demonstrated acceptable precision. Method comparison results showed acceptable agreement with an appropriate reference method.

B-272

Quantification of Ritalinic Acid and Amphetamine Related Drugs in Urine by Liquid Chromatography Tandem Mass Spectrometry

H. Liao, D. Liu, L. Lawrence, G. Baird. *University of Washington, Seattle, WA*

Background: Methylphenidate (Ritalin) is prescribed for attention deficit hyperactivity disorder (ADHD). Monitoring its major metabolite, ritalinic acid, in urine can be of value in ensuring compliance with treatment programs. To this end, an assay for ritalinic acid was incorporated into a multiplexed amphetamines confirmatory assay, and the assay was revalidated.

Methods: Patient samples, standards, and quality controls were mixed with aqueous deuterated internal standards. After mixing, the supernatant was transferred to an autosampler vial for analysis. Liquid chromatography separation was achieved by Waters XTerra C18, 2.1 x 50 mm column and Acquity UPLC system, with two mobile phase consisting of 2mM ammonium acetate/10mM acetic acid in methanol and aqueous phase. A Waters Micromass Quattro micro API tandem mass spectrometer was used for mass detection. The multiple reaction monitoring channels included quantitative and qualitative transitions of the following drugs and corresponding internal standards: amphetamine, methamphetamine, methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA/"ecstasy"), and ritalinic acid. The total analysis time was 10 min.

Results: Intraassay and interassay precision were determined by 21 repeated measures of samples containing low and high quantities of all analytes, and coefficients of variation (CV) were all less than 6%. Analytical measurement ranges (AMR) were validated by 7-point standard curves and defined as 0.1 to 100 µg/mL. The limits of quantification were defined <0.01 µg/mL by 10 repeated measures of samples containing 0.01 µg/mL of all analytes with acceptable imprecision (CV<15%). Carry over was less than 0.01% by assaying analyte-free urine directly after assaying in triplicate a sample containing 100 µg/mL of each analyte spiked into urine. The assay validation parameters for the multiplexed non-ritalinic acid assays did not differ significantly after inclusion of the ritalinic acid assay. Urine samples from seventeen patients undergoing methylphenidate treatment were assayed for ritalinic acid, with positive results observed within the AMR in 13 patients, a detection rate of 71.3% in this population.

Conclusions: A simple, multiplexed liquid chromatography-tandem mass spectrometry assay of ritalinic acid and other members of the amphetamine drug class in urine was developed, intended for use in medication compliance monitoring. Addition of the ritalinic acid assay to a pre-existing amphetamines assay did not result in any decrement in the performance of the original assay.

B-273

Performance of the Emit II Plus Oxycodone Assay on the Dimension Chemistry Systems

J. Vassallo, G. E. Siefring Jr, B. Israel, N. Morjana. *Siemens Healthcare Diagnostics, Newark, DE*

Objective: Oxycodone, a semisynthetic opioid, is used for treatment of moderate to severe pain but has a high potential for abuse and addiction. A new protocol for measurement of oxycodone has been developed on the Dimension® Chemistry Systems. The Emit® II Plus Oxycodone Assay has two cutoffs: 100 and 300 ng/mL. The assay consists of liquid, ready-to-use reagents that provide qualitative and semiquantitative results.

Methods: Precision was evaluated using the cutoff and ±25% controls according to CLSI EP5-A2. Recovery was assessed by spiking oxycodone into human urine at levels that span the assay range. Calibration stability was assessed by testing the cutoff and ±25% controls over a 31-day period. Specimens (100) for each cutoff were analyzed and the results compared to those from the Viva-E® Drug Testing System. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences with each cutoff was assessed by spiking the interferents into human urine in the presence of oxycodone at levels of ±25% of the cutoff.

Results: Qualitative repeatability CVs (rate) for the cutoff and ±25% controls ranged from 0.57 to 0.80%; within-lab CVs ranged from 0.74 to 1.03%. Semiquantitative repeatability (ng/mL) CVs ranged from 2.35 to 3.72%; within-lab CVs ranged from 4.02 to 6.58%. The overlap rate between the ±25% oxycodone controls and the cutoffs was less than 5%. Semiquantitatively, the assay quantified oxycodone-spiked samples within ±20% of nominal values. At the cutoff levels, the percent agreement of specimens between the Dimension RxL and Viva-E systems was greater than 95%. At 100 ng/mL cutoff, oxycodone reagents detect oxymorphone in urine at greater than 80%, with minimal cross-reactivity with structurally related drugs (0.01%). Potential interfering substances resulted in no false responses for the spiked ±25% controls relative to the cutoffs. A minimum of 14 days of calibration stability was demonstrated.

Conclusion: The new Emit II Plus Oxycodone Assay on the Dimension systems is a suitable screening method for urine specimens in both qualitative and semiquantitative analyses.

HOOD05162002983071

B-274

Performance Evaluation of the ADVIA Centaur Everolimus* Assay

S. Baxter[1], T. Chuang[2]. [1]*Randox Laboratories Ltd., Crumlin, United Kingdom,* [2]*Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

Background: Everolimus is a sirolimus analogue bearing a stable 2-hydroxyethyl chain substitution at position 40. Everolimus has a mechanism of action similar to that of sirolimus; both molecules bind to the intracellular FK506-binding protein (FKBP12), which subsequently binds to mTOR complex, inhibiting downstream IL-2 signal transduction. The correlation between steady-state trough concentration and area under curve (AUC) provides reliable therapeutic monitoring for everolimus exposure at the trough level. The objective of this study was to determine the initial performance characteristics of the Everolimus assay on the ADVIA Centaur® XP Immunoassay System.

Methods: The ADVIA Centaur Everolimus assay is a competitive immunoassay using direct chemiluminescent technology. In the assay reaction, free everolimus in the patient sample competes with the bound everolimus in the solid phase for binding with the acridinium ester-labeled anti-everolimus antibody. The amount of everolimus present in the patient sample is measured by an inverse relationship to the amount of relative light units detected by the system.

Preliminary performance characteristics of the assay, including inter- and intra-assay precision, linearity, limit of quantification, interfering substances, and method comparison, were determined. For precision, a total of 80 replicates were tested for each sample across 20 days. Sixty-one EDTA whole-blood samples from kidney transplant patients with everolimus concentrations of 2.3 to 24.9 ng/mL were tested using the ADVIA Centaur Everolimus assay and the LC-MS/MS Everolimus method conducted at Analytical Sciences International (UK). The relationship between methods was analyzed using Passing-Bablok regression. Interference studies were conducted with a panel of endogenous substances, as well as with potentially co-administered drugs, at concentrations of approximately 3 times the maximum recommended dosage.

Results: The ADVIA Centaur Everolimus assay demonstrated a mean %CV of 2.7 for intra-assay and 4.3 for total precision (across a sample range of 3.2 to 26.9 ng/mL). Good correlation to LC-MS/MS was demonstrated, with a slope and intercept of 1.01 and 0.08, respectively. LoD and LoQ were 0.75 ng/mL and 0.98 ng/mL, respectively. Linearity was demonstrated across a range from LoQ to 30 ng/mL. The interfering substances were tested at the recommended concentrations and found not to significantly interfere with assay results, at ≤10% difference to the control condition.

Conclusion: The resulting data demonstrates that the ADVIA Centaur Everolimus assay is an accurate and precise method of quantifying everolimus concentration in EDTA whole-blood samples compared to LC-MS/MS.

*Assay under development by Randox Laboratories Ltd. for Siemens Healthcare Diagnostics Inc. Not available for sale. Future availability cannot be guaranteed.

B-275

Performance of the Emit II Plus Oxycodone Assay on the ADVIA Chemistry Systems

B. Gabriel, G. E. Siefring Jr, B. Israel, N. Morjana. *Siemens Healthcare Diagnostics, Newark, DE*

Objective: Oxycodone, a semisynthetic opioid, is used for treatment of moderate to severe pain but has a high potential for abuse and addiction. A new protocol for measurement of oxycodone has been developed on the ADVIA® Chemistry Systems. The Emit® II Plus Oxycodone Assay has two cutoffs: 100 and 300 ng/mL. The assay consists of liquid, ready-to-use reagents that provide qualitative and semiquantitative results.

Methods: Precision was evaluated using the cutoff and ±25% controls according to CLSI EP5-A2. Recovery was assessed by spiking oxycodone into human urine at levels that span the assay range. Onboard stability was assessed by testing the assay controls over time. Specimens (100) for each cutoff were analyzed and the results compared to those from the Viva-E® Drug Testing System. Cross-reactivity with structurally related drugs was assessed at different cross-reactant concentrations. The effect of common interferences with each cutoff was assessed by spiking the interferents into human urine in the presence of oxycodone at levels of ±25% of the cutoff.

Results: Qualitative repeatability CVs (rate) for the cutoff and ±25% controls ranged from 0.44 to 0.93%; within-lab CVs ranged from 0.84 to 1.27%. Semiquantitative repeatability (ng/mL) CVs ranged from 1.69 to 6.32%; within-lab CVs ranged from 3.06 to 7.96%. The overlap rate between the ±25% oxycodone controls and the cutoffs was less than 5%. Semiquantitatively, the assay quantified oxycodone-spiked samples within ±20% of nominal values. At the cutoff levels, the percent agreement of specimens between the ADVIA 1800 Chemistry and Viva-E systems was greater than 95%. At 100 ng/mL cutoff, oxycodone reagents detect oxymorphone in urine at greater than 80%, with minimal cross-reactivity with structurally related drugs (0.01%). Potential interfering substances resulted in no false responses for the spiked ±25% controls relative to the cutoffs. Reagents are stable onboard the analyzer for up to 4 weeks.

Conclusion: The new Emit II Plus Oxycodone Assay on the ADVIA Chemistry Systemss is a suitable screening method for urine specimens in both qualitative and semiquantitative analyses.

HOOD05162002983070

B-276

Performance Evaluation of the ADVIA Centaur Sirolimus* Assay

S. Baxter[1], T. Chuang[2]. [1]*Randox Laboratories Ltd., Crumlin, United Kingdom,* [2]*Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

Background: Sirolimus (rapamycin) is a lipophilic, macrocyclic lactone compound originally isolated from *Streptomyces hygroscopicus* with an inhibitory effect on the mTOR pathway. It was approved for the prophylactic treatment of acute rejection in renal transplant patients. When given to transplant patients in combination with cyclosporine and corticosteroids, there is a reported reduction in the incidence of acute organ rejection without incurring an increase in overall adverse events. However, the therapeutic range for sirolimus level is quite narrow. Therefore, it is necessary to monitor the drug levels as accurately as possible to reduce the risk of organ rejection or elevated toxicity for the patient. The objective of this study was to determine the initial performance characteristics of the Sirolimus assay on the ADVIA Centaur® XP Immunoassay System.

Methods: The ADVIA Centaur Sirolimus assay is a competitive immunoassay using direct chemiluminescent technology. In the assay reaction, free sirolimus in the patient sample competes with the bound sirolimus in the solid phase for binding with the acridinium ester-labeled anti-sirolimus antibody. The amount of sirolimus present in the patient sample is measured by an inverse relationship to the amount of relative light units detected by the system.

Preliminary performance characteristics of the assay, including inter- and intra-assay precision, linearity, limit of quantification, interfering substances, and method comparison, were determined. For precision, a total of 80 replicates were tested for each sample across 20 days. Thirty-one EDTA whole-blood samples from kidney transplant patients with sirolimus concentrations from 2.4 to 27.4 ng/mL were tested using the ADVIA Centaur Sirolimus assay and the LC-MS/MS Sirolimus method, conducted at Analytical Sciences International (UK). The relationship between methods was analyzed using Passing-Bablok regression. Interference studies were conducted with a panel of endogenous substances, as well as with potentially co-administered drugs at concentrations of approximately 3 times the maximum recommended dosage.

Results: The ADVIA Centaur Sirolimus assay demonstrated a mean %CV of 3.21 for intra-assay and 5.43 for total precision (across a sample range from 1.66 to 25.36 ng/mL). Good correlation to LC-MS/MS was demonstrated, with a slope and intercept of 1.095 and 0.149, respectively. LoD and LoQ were 0.32 ng/mL and 0.94 ng/mL, respectively. Linearity was demonstrated across a range from LoQ to 30 ng/mL. The interfering substances were tested at the recommended concentrations and found not to significantly interfere with assay results, at ≤10% difference to the control condition.

Conclusion: The resulting data demonstrates that the ADVIA Centaur Sirolimus assay is an accurate and precise method of quantifying sirolimus concentration in EDTA whole-blood samples compared to LC-MS/MS.

*Assay under development by Randox Laboratories Ltd. for Siemens Healthcare Diagnostics Inc. Not available for sale. Future availability cannot be guaranteed.

B-277

Everolimus Assay* with Automated Pretreatment for the Dimension Chemistry Systems

J. Li, C. Tyler, T. Q. Wei. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: An everolimus (EVRO) assay* with automated pretreatment for the Dimension® Integrated Chemistry Systems is currently under development. The aim of this assay is to provide confidence in patient results with improved productivity for complete transplant-patient care.

Methods: The EVRO assay is based on ACMIA technology. The principle and operation of the EVRO assay are as follows: A pretreatment reagent is added to a reaction vessel on the Dimension system. Next a sample of whole blood containing everolimus is added. The blood and pretreatment reagent react to ensure the lysis of the whole blood and the release of bound drug. An anti-everolimus antibody-β-galactosidase conjugate is added next and allowed to react with everolimus from the patient sample. Finally, pre-decorated chrome particles coated with an everolimus analog are added and allowed to bind the unreacted conjugate. The everolimus-bound conjugate does not bind to the chrome but remains in the supernatant when a magnetic field is applied to the reaction mixture. The everolimus-bound conjugate is detected by transferring the supernatant from the reaction vessel to a photometric cuvette, where the enzyme tag is detected using a sensitive chromogenic substrate.

Results: The assay uses a 12 µL sample size, and the calibration is stable for 30 days. Time to first result is 15 minutes, and results are linear to 30 ng/mL. The assay showed a functional sensitivity of <1 ng/mL. Repeatability and within-lab reproducibility CVs were tested using patient whole-blood pools and QC samples ranging from 2.4 to 25 ng/mL and were found to be less than 5 and 6% respectively. A close relationship was observed between the Dimension EVRO assay and an LC-MS/MS reference method: EVRO = 1.06(LC-MS/MS) - 0.20; r = 0.97 (n = 170, range: 1.0 to 30.0 ng/mL).

Conclusion: The Dimension Everolimus assay demonstrates acceptable precision, accuracy, and turnaround time for everolimus measurement on the Dimension System. * Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

B-278

A Rapid Plate-Format Label-Free Immunoassay for Quantitation of Monoclonal Antibody Drugs and Detection of Anti-Drug Antibodies in Serum Samples

Y. Luo, A. H. Wu, K. L. Lynch. *University of California San Francisco, San Francisco, CA*

Background: Monoclonal antibody (mAb) drugs have been among the top-line sales therapeutics. Their initial response rates vary and often an initial response is lost over time. Therefore, therapeutic drug monitoring (TDM)-guided dose optimization based on measuring serum concentrations of mAb drugs in individual patients is in demand. Besides the concentration of active mAb drugs, the quantitation of antidrug antibodies (ADA) is also of interest because the formation of ADA can result in nonresponse in patients.

In the clinical laboratory setting, TDM of mAb drugs has been measured by ELISA, RGA, LC-MS, etc. On the other hand, immunoassays based on label-free technologies have great advantages in implementing TDM of mAb drugs. The label-free technologies detect of antibody-antigen binding without attaching a reporter to the immunocomplex, which allows for binding kinetics-based quantitation and facilitates

rapid quantitative immunoassays, i.e. within 5 minutes of assay time. A rapid plate-format label-free immunoassay was established using a novel label-free technology thin-layer interferometry (TLI) technology for quantitation of mAb drugs adalimumab (ADL) and infliximab (IFX).

Methods: The mAb drug was spiked in drug-free serum to prepare a series of mAb drug calibrators. The label-free immunoassay was carried out in a Gator TLI analyzer (Probe Life, Palo Alto, CA). Quantitation of active mAb drug: Biotin-TNF-α was immobilized to streptavidin (SA) sensors. The samples were 10-fold diluted in PBS with Tween 20 and BSA. The coated SA sensors were applied to the samples. The initial binding rate of active mAb drug was measured for 5 min. Detection of ADA: The mAb drug was immobilized to anti-human Fc (HFC) sensors. The coated HFC sensors were applied to the samples. The binding equilibrium point of ADA was measured for 5 min.

Results: The label-free immunoassay for active mAb drug was validated. Imprecision (CV) was determined at three levels: 6.8% at 5.0 μg/ml, 8.9% at 25 μg/ml, 5.3% at 50 μg/ml. Accuracy was measured by bias: -13% at 5.0 μg/ml, 4.8% at 25 μg/ml, -0.3% at 50 μg/ml. Limit of quantitation (LOQ) is 2.5 μg/ml (CV 8.5%, Bias 6.3%). Dynamic range is from LOQ to 50 μg/ml.

In an exploratory experiment, two serum samples for TDM of ADL and one serum sample for TDM of IFX were tested for active mAb drug concentrations. The results are listed as follows (ELISA results in parenthesis). ADL sample 1: below LOQ (ELISA not detected); ADL sample 2: 9.5 μg/ml (ELISA 7.79 μg/ml); IFX sample: 24.6 μg/ml (24.33 μg/ml). The ADL samples were also tested for ADA. ADL sample 1 was positive and ADL sample 2 was negative (same as qualitative ELISA).

Conclusion: The label-free immunoassay based on TLI technology showed good performance in quantitation of active mAb concentrations and detection of ADA in serum samples. The results were consistent with the conventional ELISA method. Employing binding kinetics-based quantitation, the immunoassay only takes 5 minutes. Provided the therapeutic trough concentration of ADL is 5 ~ 8 μg/ml and that of IFX is 3 ~ 7 μg/ml in serum, the sensitivity of label-free immunoassay meets the need for TDM of mAb drugs.

B-279

Development and Validation of a Free Phenytoin Assay on an Automated Chemistry Analyzer

N. Boyert, X. Zhang, M. Mitro, X. Tang, A. J. McShane. *Cleveland Clinic, cleveland, OH*

Background: Phenytoin is an anticonvulsant drug, used primarily to control seizures in patients with grand mal epilepsy, cortical focal seizures, and temporal lobe epilepsy. Phenytoin is approximately 90% protein bound once absorbed, however the degree of protein binding can be influenced by co-medications or other conditions. The pharmacological effect of phenytoin is dependent on the concentration of unbound (free) phenytoin in the blood. Therefore, a method was developed and validated to measure the free phenytoin levels in serum to better assess adequate therapy and toxicity. **Methods:** Separation of the non-protein bound phenytoin was achieved via ultrafiltration through a molecular weight cut-off filter (30 kDa, Millipore). A method for free phenytoin measurement was developed on the automated cobas chemistry analyzers (c502, Roche Diagnostics) by modifying a total phenytoin assay (PHNY2, Kinetic Interaction of Microparticles in Solution, Roche Diagnostics). Assay performance characteristics were established including analytical measuring range, analytical sensitivity, analytical specificity, carryover, precision, method comparison, stability, diluent mixing study, and maximum dilution. **Results:** *Analytical Measurement Range/Analytical Sensitivity* AMR for free phenytoin was established with triplicate analysis at 6 levels, 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 μg/mL. Reference standard material (Cerilliant) was spiked into pooled patient filtrate with recovery ranging from 96.7 - 111.7%. The lower limit of quantitation was determined to be 0.1 μg/mL. *Analytical Specificity* This assay was found to be affected by hemolysis and lipemia, showing a decrease of greater than 20% of the expected result at a serum index of 500 and 333, respectively. The assay was not found to be affected by icterus (<20% percent difference). *Carryover* No carryover (<0.1 μg/mL) was observed at a concentration 10 times the upper limit of the AMR. *Precision* Intraday precision coefficient of variation (CV) ranged from 2.5-10.0% [standard deviations (SD) from 0.04-0.07 μg/mL] and the interday precision CVs ranged 2.5-11.4% (SDs from 0.06-0.07 μg/mL) at concentrations of 0.4 and 2.9 μg/mL. *Method Comparison* Forty samples were compared to the free phenytoin assay on the Integra 800 (Roche Diagnostics). The correlation coefficient was 0.9962, with a slope of 1.112, and intercept of -0.04. *Stability* Unfiltered samples, removed from cells and stored tightly capped, were found to be stable (<0.3 μg/mL difference from time 0) for 7 days at room temperature and 2-8 °C and 2 weeks at -20 °C. Once filtered, stability at room temperature, when left open to the air, was found to be 1 hour (percent difference <10%). *Mixing Study* A mixing study was performed to determine an appropriate diluent to extend the reportable range. Saline was found to be acceptable with an average percent difference of 0.6%. *Maximum Dilution* A 1:2 dilution was determined to be acceptable (percent difference <3%). **Conclusion:** This assay has been validated and shown to be an accurate and precise method of therapeutic drug monitoring for patients being treated with phenytoin. Further, the method was developed on a chemistry analyzer to allow expeditious turnaround time in a core laboratory.

B-280

Performance Evaluation of the Emit II Plus Oxycodone Assay on the Beckman Coulter AU680 System

A. Walters, B. Israel, G. E. Siefring. *Siemens Healthcare Diagnostics Inc., Newark, DE*

Background: Oxycodone is a semisynthetic opioid analgesic prescribed for the relief of moderate to severe pain with high potential for addiction and abuse. The Emit® II Plus Oxycodone assay has cutoffs of 100 and 300 ng/mL. The assay consists of ready-to-use liquid reagents that provide qualitative and semiquantitative results. A new protocol for the measurement of oxycodone has been developed on the Beckman Coulter AU Systems. The data presented in this study was generated on the Beckman Coulter AU680 System. Application protocols have also been developed for the Dimension®, Dimension Vista®, ADVIA® Chemistry, and Atellica® CH systems.

Methods: Precision was evaluated at the cutoffs and ±25% controls according to CLSI EP5-A3. Analytical recovery was studied by spiking oxycodone into human urine at levels that span the assay range (50-1000 ng/mL). Specimens (100 per cutoff) were analyzed and the results compared to those of the Viva-E® Drug Testing System. Cross-reactivity with structurally-related drugs was assessed at different cross-reactant concentrations. The effect of common interferents was assessed by spiking the interferents into human urine in the presence of oxycodone at levels of ±25% of the cutoffs. On-instrument stability was assessed by testing the assay controls over time.

Results: Evaluation of precision demonstrated qualitative repeatability CVs (rate) for all levels that ranged from 0.48 to 0.63%, and within-lab CVs ranged from 0.91 to 1.14%. Semiquantitative repeatability CVs (ng/mL) ranged from 2.38 to 3.83%, and within-lab CVs ranged from 5.64 to 8.87%. Semiquantitatively, the assay quantified oxycodone-spiked samples between 50 and 400 ng/mL for the 100 cutoff curve and 100-1000 ng/mL for the 300 cutoff curve within ±20% of nominal values. The percent agreement of specimens between the assay run on the AU680 System and Viva-E Drug Testing System was 100% at both the 100 ng/mL and 300 ng/mL cutoffs. The assay demonstrated 88% detection of oxymorphone in urine relative to oxycodone at the 100 ng/mL cutoff. The assay demonstrated minimal cross-reactivity to structurally-related opioids. Potentially interfering substances gave acceptable results relative to the 100 and 300 ng/mL cutoffs. The reagents were stable onboard the AU680 System for a minimum of 4 weeks.

Conclusion: The Emit II Plus Oxycodone assay on the Beckman Coulter AU680 System is a suitable screening method for urine specimens for both qualitative and semiquantitative analyses.

B-281

Large-Scale Analysis of Electronic Health Record Data Enables Systematic Discovery of Cross-reactivity in Urine Drug Screening Immunoassays

J. M. Colby, J. J. Hughey. *Vanderbilt University School of Medicine, Nashville, TN*

Background: Urine drug screening immunoassays (UDS) are known to detect a variety of off-target compounds. This cross-reactivity is one reason that presumptively positive screening results should be confirmed by a more specific technique. However, during the period before confirmatory results are available, cross-reactivity could lead healthcare providers to make erroneous assumptions about drug exposure. Hence, a comprehensive list of cross-reactive substances would be highly useful. Unfortunately, the case-based approach by which cross-reactivity is typically discovered is inefficient and unlikely to reveal cross-reactivity caused by rarely-used medications. We sought to systematically discover cross-reactive substances using a large-scale statistical analysis of data from electronic health records (EHRs).

Methods: The EHR data were from the Synthetic Derivative, a de-identified database of clinical data from Vanderbilt University Medical Center. We extracted results for UDS and confirmation assays collected since 2014. For each person in the dataset, we extracted drug exposures (primarily medication orders) that occurred between 1 and 30 days prior to that person having a screen-confirmation result. We used RxNorm to

map each drug to its active ingredient(s). We defined a false positive as a presumptive positive screen followed by a negative confirmation. To quantify the association of a given ingredient with false-positive results on a given screening assay, we calculated the odds ratio (OR) and the corresponding 95% confidence interval. An odds ratio of 2 means that the odds of a false positive (as opposed to a negative screen) on that assay went up by a factor of 2, if the person had a documented exposure to that ingredient between 1 and 30 days prior. We calculated p-values using a chi-squared test and Fisher's exact test. We evaluated each compound's cross-reactivity by spiking a reference standard into drug-free urine and testing the spiked samples on the target immunoassay on an Abbott Architect c16000 chemistry analyzer.

Results: Our dataset contained 674,195 screen-confirmation result pairs from 37,168 individuals and ten different screens, of which 6,512 result pairs were false positives. Altogether, we tested for a statistical association between false positive results and drug exposure for 647 ingredients. Here we describe preliminary findings for the amphetamines screen, which had 1,254 false positives (27.6% of presumptive screen results). Of the top ten predicted cross-reactants for the amphetamines assay, two were assay targets (dextroamphetamine and lisdexamphetamine) and three were known cross-reactants (trazodone, mexiletine, and procainamide). We tested the two ingredients with the highest odds ratios, methyldopa (OR 15.4) and ceftaroline fosamil (OR 57.4). We were unable to confirm the cross-reactivity of methyldopa, which is extensively metabolized prior to excretion in urine. Ceftaroline fosamil, however, had a confirmed cross-reactivity of 0.9% at clinically relevant concentrations of less than 100 µg/mL.

Conclusion: Our preliminary findings suggest that integrating lab results and drug exposures from electronic health records can identify compounds that cross-react with urine drug screening immunoassays. Our unbiased approach may enable laboratorians to build and maintain a comprehensive catalog of cross-reactants.

B-282

Detection of Cannabinoid Compounds in Dried Blood Spots by Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS)

H. A. Paul, H. Awad, H. Sadrzadeh, J. M. Boyd. *Alberta Public Laboratories, Calgary, AB, Canada*

Background: Dried blood spots (DBS) have been the specimen of choice for newborn screening for several decades as they can be collected without phlebotomy and require only a small amount of blood. DBS have also become a specimen of interest for toxicology testing and therapeutic drug monitoring. With the increased of use of cannabinoids in pain management, there is interest in monitoring cannabinoids in blood to follow therapy. DBS are an ideal specimen type for this patient population as samples could be collected in clinic or at home, saving the patient a trip to the laboratory. The objective of this study is to develop a method to detect various cannabinoids and their metabolites in DBS.

Methods: Working solutions containing seven target compounds (tetrahydrocannabinol, THC; 11-nor-9-carboxy-THC, THC-COOH; 11-hydroxy-THC, 11-OH-THC; Cannabidiol, CBD; Cannabinol, CBN; tetrahydrocannabivarin, THCV; tetrahydrocannabivaric acid, THCVA) were prepared as a mix of 10 ug/mL in methanol. Spiked DBS were prepared by adding 50 µL of target compounds to 950 µL EDTA whole blood to achieve a final concentration of 500 ng/mL (20 ng/DBS). Spiked sample (40 µL) was applied to Whatman 903 Protein Saver cards and dried overnight. The entire blood spot was cut out from the card and cannabinoids were extracted using 1 mL of methanol containing 1 ug/mL deuterated internal standards by vortexing for 5 mins. Extracts were centrifuged for 10 mins and the methanol evaporated using nitrogen. The residue was dissolved in a Mobile A, filtered, and the filtrate transferred to a glass vial. Cannabinoids were detected using an ultra-high performance liquid chromatography system (Waters Acquity UPLC) coupled to a triple quadrupole mass spectrometer (Waters Xevo-TQ-XS). Reversed-phase chromatography was performed using a Restek Raptor Biphenyl column (2.7um particles, 100 x 2.1mm) with a linear gradient elution using Mobile A (5 mmol/L ammonium formate, pH 3.0) and Mobile B (0.1% formic acid in acetonitrile) at a flow rate of 0.350 mL/min. Mobile A was maintained at 99% for 4.50 min, decreased to 60% until 7.00 mins, decreased to 0% until 8.25 mins, and then increased to 99% for re-equilibration. Analytes were detected using multiple reaction monitoring (MRM) mode via positive (THC, 11-OH-THC, CBD, CBN, THCV, THCVA) and negative (THC-COOH) electrospray ionization. Mass spectrometer settings were optimized for each analyte: source gas pressure, 90-100 psi; argon collision gas, 7 psi, and cone voltage, 2-52V.

Results: All target compounds were selectively detected and separated with a run time of 9 mins. The compounds eluted in the following order: THCVA, 11-OH-THC, THC-COOH, CBD, THCV, CBN, and THC. The isomers CBD and THC were monitored using two identical MRM transitions (315.0>193.0; 315.0>123.0) and the compounds distinguished by retention time. Optimization of the extraction protocol and establishment of calibration curves is expected to improve the sensitivity of this method.

Conclusion: Our preliminary results show that DBS can be used to detect several cannabinoids and metabolites simultaneously. It is an easy and economical approach to monitor drug therapy. Results of the optimized extraction method and method validation will be presented at the annual meeting.

B-283

Use of Illicit Drugs among Patients of Pain Management Clinics of Texas

S. Mansani, D. Ponds, C. Cobb, S. Alsaab, M. Haidari. *Elite Medical Laboratory Solutions, Tomball, TX*

Background: Clinicians in pain management clinics treat patients with chronic pain or substance use disorders. Urine drug testing performed in clinical toxicology laboratories is used by clinicians to monitor patients` compliance to therapy and to detect use of illicit drugs by patients. Detection of illicit drugs in the patient`s urine leads to dire consequences for patients such as termination of treatment plan. Although use of illicit drugs is common between these patients, no prior data is available about the frequency of illicit drugs use. We conducted this study to investigate prevalence of use of illicit drugs among patients that are monitored by clinical toxicology laboratories via urine drug testing.

Methods: In a retrospective study the presence of illicit drugs (tetrahydrocannabinol, cocaine, heroin, methamphetamines, 3,4-methylenedioxy-methamphetamine (MDMA), 3,4-methylenedioxy-amphetamine (MDA), phencyclidine), and 54 other commonly tested drugs in urine specimens of 24,619 patients were evaluated. The urine specimens were collected between August 2016 to January 2019 from patients of pain management clinics located in Texas. Confirmatory urine drug testing was performed by a validated dilute and shoot liquid chromatography mass spectrometry method. The presence of cocaine and heroin was evaluated by detection of their urinary metabolites, benzoylecgonine and 6-mono acetyl morphine (6-MAM), respectively.

Results: At least one illicit drug was detected in urine of a total of 2,922 patients (11.84 % of all evaluated patients). The chance of using illicit drugs were almost 2.5 times higher for individuals younger than 40 years old and 35% higher for males then females. Tetrahydrocannabinol was the most frequently detected illicit drugs among the tested urine specimens (9.15%). Cocaine, heroin, methamphetamine, MDMA, MDA, and phencyclidine were detected with frequency of 1.24%, 0.13%, 0.87%, 0.02%, 0.01%, and 0.02% of urine specimens, respectively. In 291 patients (approximately 10% of positive patients) 2 or more illicit drugs were detected. Methadone or its metabolite, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) or buprenorphine or its metabolite, norbuprenorphine were detected in urine of 1,095 patients and around 20% of this population (217 patients) simultaneously used one or more illicit drugs.

Conclusion: Tetrahydrocannabinol, cocaine, heroin, methamphetamines, MDMA, MDA, and phencyclidine are the most common illicit drugs detected in urine of patients from pain management clinics in Texas.

B-284

Evaluation of Drug Adsorption to the PIVO™ Needless Blood Collection Device

C. D. Koch[1], J. Meyers[2], J. H. Noguez[2]. [1]*Cleveland State University, Cleveland, OH,* [2]*University Hospitals Cleveland Medical Center, Cleveland, OH*

Objectives

The use of a needle-free blood collection device is highly desirable to avoid repeated venipuncture in patients requiring frequent diagnostic laboratory testing. Blood collections from peripheral intravenous catheters (IVCs) can offer several benefits to these patients and can be used for most routine laboratory testing with the exception of therapeutic drugs due to drug adsorption to the plastic. PIVO™ is an FDA-cleared, single-use, needless blood collection device (Velano Vascular Inc.) that connects to the hub of a peripheral IVC extension set and deploys an extendable cannula through the IVC into an area of undisturbed flow in the vein. Previous studies have demonstrated that the sample quality generated by this device is equivalent to venipuncture for several common chemistry analytes but did not include therapeutic drugs. The objective of this study was to evaluate the interaction between the PIVO™ blood collection device and several therapeutic drug classes with regard to adsorption potential, including immunosuppressant (tacrolimus, sirolimus, and cyclosporine), anti-epilep-

tic (vancomycin, valproic acid, levetiracetam, lamotrigine, phenytoin, and phenobarbital), anti-arrythmic (digoxin) and anti-neoplastic (methotrexate) drugs.

Methods

Residual clinical patient samples collected via traditional venipuncture were divided into two aliquots. The first aliquot was used as a control as it was not exposed to the PIVO™ device. The second was pulled through a PIVO™ device into a VACUETTE® Blood Collection Tube (Greiner Bio-One) without additive to simulate the flow conditions and exposure time experienced in an actual sample collection. Drug levels were determined pre- and post-PIVO™ exposure in duplicate using a Beckman AU5800 chemistry analyzer (Beckman Coulter) or an Abbott Architect i1000s immunoassay analyzer (Abbott Diagnostics). A minimum of twenty samples were evaluated per analyte, spanning the analytical measurable range but focusing on the therapeutic range. The differences of the pre- and post-exposure mean values were determined and analyzed using linear regression and bias plots. Statistically significant bias was defined as P-value <0.05.

Results

Linear regression analysis of the pre- versus post-PIVO™ exposure drug levels yielded slopes ranging from 0.95 - 1.03, intercepts ranging from -0.39 to 13.02, and correlation coefficients of 0.96 - 1.00. A statistically, but not clinically, significant mean positive bias of ~ 0.2 ng/mL was observed for both tacrolimus (p=0.0200) and sirolimus (p=0.0477). None of the remaining drugs demonstrated statistically significant biases. Of the 10 drugs with established therapeutic ranges, all had >90% concordance in the clinical interpretation of the pre- vs post-PIVO exposure drug values with the exception of phenytoin with 87% (26/30). All discordant samples were near the therapeutic cutoffs and had biases within the expected imprecision levels for the respective assay.

Conclusions

This study demonstrates that short-term exposure of drugs to the PIVO™ cannula, such as experienced in a normal blood collection, does not significantly affect TDM assay results. The data suggests that PIVO™ is a viable, needle-free alternate to venipuncture for therapeutic drug blood testing potentially allowing for a paradigm shift in TDM sample collection. Further studies directly comparing TDM results of patient blood samples collected by PIVO™ versus venipuncture are warranted and underway.

B-285

Multi-Laboratory Evaluation of the Thermo Scientific™ QMS™ Plazomicin Immunoassay for Therapeutic Drug Monitoring (TDM) in Treatment of Serious Bacterial Infections Due to Multi-Drug Resistant (MDR) *Enterobacteriaceae*

E. Palavecino[1], Z. Lin[1], A. Greggs[2], K. Garrison[1], J. Huang[3], A. Tsai[3], J. Donaldson[3], L. Ye[3], K. Jamison[4], T. Rigl[3], T. Prestigiacomo[3], S. Wong[1], D. Koch[2]. [1]*Wake Forest University School of Medicine, Winston Salem, NC,* [2]*Emory University and Grady Memorial Hospital, Atlanta, GA,* [3]*Thermo Fisher Scientific, Fremont, CA,* [4]*Achaogen, San Francisco, CA*

Background: The rapid spread of multi-drug resistant (MDR) bacteria is rendering current therapies less effective. Plazomicin (ZEMDRI®) is an aminoglycoside approved for the treatment of patients with complicated urinary tract infections (cUTI) including pyelonephritis due to MDR Enterobacteriaceae. Plazomicin is synthesized from sisomicin and contains structural modifications allowing it to maintain activity in the presence of common aminoglycoside-modifying enzymes that inactivate other commercially available aminoglycosides. The QMS Plazomicin Immunoassay is a homogeneous particle-enhanced turbidimetric inhibition immunoassay intended for the quantitative determination of plazomicin in human K_2 EDTA plasma on automated clinical chemistry analyzers. The assay results obtained should only be used as an aid in the management of patients with complicated urinary tract infection (cUTI) receiving plazomicin therapy. The objective of these studies was to evaluate the performance of this assay for its potential as an aid for therapeutic drug monitoring (TDM) in the emerging practice of personalized medicine. **Methods**: The QMS Plazomicin Immunoassay consists of two reagent components, namely a monoclonal anti-plazomicin antibody and plazomicin-coated microparticles, six calibrators providing a calibration range from 0 to 40.0 µg/mL and tri-level controls. The immunoassay is based on competition between free plazomicin present in a sample and plazomicin derivative coated onto microparticles for antibody binding sites. The plazomicin-coated microparticle reagent is rapidly agglutinated in the presence of the anti-plazomicin antibody reagent. When a sample containing plazomicin is added, a concentration-dependent agglutination inhibition curve is obtained. The QMS Plazomicin Immunoassay performance characteristics were evaluated at three laboratories with Beckman Coulter® AU680 analyzers using K_2 EDTA plasma samples obtained from patients treated with plazomicin. The analytical studies were based on CLSI guidelines. **Results**: The assay range was determined to be 0.8-34.0 µg/mL. The limit of quantitation (LoQ) was determined to be ≤0.8 µg/mL for K_2 EDTA plasma samples. The assay was linear with less than 10% bias for plazomicin concentrations between 0.8-34.0 µg/mL, which includes the expected therapeutic ranges. Assay precision and accuracy in controls containing 2.5, 8.0, and 30.0 µg/mL of plazomicin, K_2 EDTA plasma spiked with 2.5, 8.0, 15.0, and 30.0 µg/mL of plazomicin, and 2.5, 8.0, and 30.0 µg/mL plazomicin patient pools were determined by testing twice per run, two runs per day for 20 days (5 days for patient pools). These experiments yielded within-run precision between 1 and 7% CV and total precision between 2 and 8% CV. Total repeatability was 1 to 5% CV, and the reproducibility ranged from 3 to 8% CV with total error of less than 20%. The combined three-laboratory method results for the QMS Plazomicin Immunoassay were compared to a validated LC-MS/MS reference method using clinical plazomicin plasma samples and yielded a Passing-Bablok equation of y = 1.0x + 0.41 (n=134) and a correlation coefficient of greater than 0.983. The calibration curve was stable for at least 7 days. **Conclusions**: The Thermo Scientific QMS Plazomicin Immunoassay enables measurement of plazomicin with acceptable precision and accuracy between 0.8 - 34.0 µg/mL. This project has been funded in part with Federal funds from the Biomedical Advanced Research and Development Authority (BARDA).

B-286

Fast and Reliable Method for Detection of Phenylglyoxylic Acid in Urinary Samples to Monitor Styrene Exposure

E. K. Sugawara, J. S. Ratero, D. R. Ramadan, M. De Martino, S. S. Andrade, S. Tufik. *Associação Fundo de Incentivo a Pesquisa (AFIP - Medicina Diagnóstica), São Paulo, Brazil*

Background: The phenylglyoxylic acid (PGA) is a metabolite in the urine samples that indicate excessive occupational exposure to the styrene. Human exposure to PGA occurs mainly by inhalation in occupational settings. It is toxic to the central nervous system, an irritant of mucous membranes and can be a cause of gastrointestinal, liver and kidney disorders. **Methods:** We used high performance liquid chromatograph consisting of autosampler, quaternary pump and UV detector with variable wavelength. Detection was accomplished at 240nm. For the chromatographic separation was used analytical column and maintained at 30°C during the analysis. The mobile phase was water and acetonitrile (88:12,v/v) with a flow rate of 0.5 ml/min. The linearity was observed in the expected concentration range. Urine was used as a biological matrix for the study and PGA was evaluated as a standard. **Results:** The linearity was analyzed using enriched urine samples with PGA from 15.63 to 1000.00 mg/L, then the samples were evaluated by six times, each. The intra-assay precision was expressed by average coefficient of variation (CV%) for urine sample analyzed from seven different concentration in six times, each. To determine inter-assay (CV%) mean were analyzed three different concentrations over three days. The accuracy of the method was verified by analyzing samples of known concentration and expressed as percentage. The retention time was 3.5 min and for the final analysis was 7 min. The use of water and acetonitrile in the mobile phase ensured simplicity and low cost in the exam. The coefficient of determination (R^2) was 0.99959. The intra, inter-assay precision and accuracy are shown in table. **Conclusion:** The method was efficient and robust to determine the PGA dosage in the control of occupational exposure to styrene and could be used as a reliable tool to help the prevention and control of the employee exposure in the workplace.

Concentration mg/L	Precision (%)		Accuracy (%)	
	Intra-Assay n=6	Inter-assay n=18	Intra-Assay n=6	Inter-assay n=18
15.63	8.3	N.D.	97.6	N.D.
32.25	12.6	N.D	102.6	N.D.
62.50	6.2	5.8	104.2	102.7
125.00	2.4	2.2	98.7	98.6
250.00	2.1	3.1	101.4	101.3
500.00	3.2	N.D.	99.0	N.D.
1000.00	3.0	N.D.	100.2	N.D.

N.D.: Not determined

B-287

Evaluating the Impact of an In House Therapeutic Drug Monitoring for Antifungal Drugs on the Length of Hospital Stay

A. Thombare, K. Leonard, M. Smith. *Beaumont Health System, Southfield, MI*

Objective: To determine the influence of in house therapeutic drug monitoring for antifungal drugs on the length of hospital stay.

Background: Systemic fungal infections in hospitalized patients are treated with oral or intravenous antifungal medications. Therapeutic drug monitoring (TDM) has been recommended and increasingly utilized to guide the management of patients with these infections. Many TDM services are only available in reference laboratories. In the past, all samples for anti-fungal TDM from Beaumont Health System were sent to an outside reference laboratory with a turn-around time of five to seven days. We have developed and implemented an assay by Liquid chromatography-mass spectrometry method at Beaumont Toxicology Laboratory, Royal Oak. The turn-around time for this test is ≤ 24 hours. The purpose of this study was to evaluate the newly implemented in-house TDM for antifungal medications can shorten the length of stay for patients admitted in the hospital.

Methods: Starting January 8, 2019, in-house TDM assay was implemented for antifungal medications including Voriconazole, Itraconazole, Hydroxy-itraconazole, Posiconazole, and fluconazole. We collected the data for patients before (baseline data) and after (intervention data) the implementation of in-house testing. These data included patient demographics, medication and length of hospital stay. Primary outcome was length of hospital stay (LOS) per patient in days. The baseline and intervention outcomes were compared.

Results: Between July, 2018 and December, 2018 average baseline LOS was 25 days. Average LOS for patients after the implementation of in house TDM was 21.3 days.

Conclusions: An implementation of in-house therapeutic drug monitoring for antifungal drugs reduced the turn-around time as well as length of hospital stay.

B-288

Toxicology Screening by Mass Spectrometry for Emergency Intoxication

S. Li[1], Y. Lu[1], W. Wang[1], C. Tong[2]. *[1]Shanghai Xuhui Central Hospital, Shanghai, China, [2]Zhongshan Hospital, Fudan University, Shanghai, China*

Background: A great challenge in managing emergency intoxication is prompt identification of the toxins. A versatile, fast, and reliable method is critical for clinical toxicology laboratories to aid in prompt diagnosis of suspicious emergency intoxication cases.Due to the lack of CFDA approved methods and advance technologies needed in the clinical laboratories, clinical toxicology screening service currently is rarely available for emergency departments in China. **Methods:** A liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF/MS) method was developed and validated in our laboratory with coverage of 2231 substances within 20 min per runfor various sample types, including blood, plasma, urine, saliva, gastric washing solution. For those samples with positive screening results, a liquid chromatography coupled to quadrupole mass spectrometry (LC-QQQ/MS) method was applied to confirm and quantify 235 frequently encountered drugs and poisons, including sedative-hypnotics, antipsychotics, analgesics, pesticides and rodenticides, drugs of abuse. The performance of the screening and quantitative MS methods were validated by participating in urine toxicology proficiency test programs provided by CAP.The MS methods were successfully applied to toxicology screening and quantification for emergency Intoxication patients.

Results: After a simple preparation, samples were screened by an LC-QTOF/MS with IDA product ion scanbased on retention time and exact mass acquired from molecular ions and fragment ions, as well as the structure matching. After positive screening, thesampleswere further tested by an LC-QQQ/MS to confirm and quantify the target drugs or poisons. For LC-QTOF/MS, the limit of detection was 10-100 ng/mL. For LC- QQQ/MS, the accuracy was 88-113% and precision was less than 10% with linearity curve ranging from 10-1000 ng/mL. The results for these determinations were reported 2-4 hours of sample receipt. All the results from CAP urine toxicology PT program were acceptable for consecutive three events. From April 2017 to November 2018, 105 suspected intoxicationcases from the EmergencyDepartments of our hospital and other hospitals located in Shanghai, China were screened. Fifty-five percent (58/105) cases were tested positive for toxic drugs or poisons, of which drug overdose, pesticide poisoning, and other poisons accounted for 79% (46/58), 17% (10/58), and 3% (2/58), respectively. Here we present three intoxicationcases of which toxins were identified by the mass spectrometry methods. (1) A 61-year-old man with depression was found unconscious in his residence hallway. Two hours later, he was transported to Emergency Department, and was eventually found taking an excess of zolpidem (a hypnotic drug) for attempted suicide. (2) A 49-year-old man was admitted to Emergency Department with a scrotum ulcer. He died later and was suspected of chronic poisoning. After toxicology screening, he was found be poisoned by fatal paraquat(a herbicide). (3) A 49-year-old man was vomiting, in coma, and with breathing difficulties after drinking a tea with unknown origin of wild fruit and was diagnosed to be rotenone poisoning.

Conclusion: Poison screening is one of the indispensable diagnostic tools for emergency intoxication cases. We demonstrated that LC-QTOF/MS and LC-QQQ/MS offer extensive coverage and accurate quantification for toxic drugs or poisons with a simplified sample preparation, making reliable and prompt diagnosisof clinical intoxication a reality.

B-289

Association of *Glutathione S-Transferase*Genes (GSTM1 and GSTT1) with Blood Lead Levels in Occupationally Lead-Exposed Workers: A Pilot Study

S. K. Datta[1], .. Himani[2], D. Sharma[3], A. A. Mahdi[3], B. Karunanand[4]. *[1]All India Institute of Medical Sciences, New Delhi, India, [2]All India Institute of Medical Sciences, Rishikesh, India, [3]King George Medical University, Lucknow, India, [4]SGT Medical College Hospital & Research Institute, Gurugram, India*

Background:

Polymorphisms in several genetic loci including glutathione S-transferase (GST) supergene family are believed to alter the toxicokinetics and/or toxicodynamics of lead. This may potentially lead to accumulation of lead within the body.

Methods:

In this study we investigate the association of *GST-mu1* (*GSTM1*) and *GST-theta1* (*GSTT1*) insertion-deletion (InDel) gene polymorphisms with blood lead levels in subjects with occupational lead exposure and comparing it with subjects who are not occupationally exposed. For this cross-sectional study we recruited 100 adult males who were apparently healthy after institutional ethical clearance and informed consent. 50 of them were occupationally exposed to lead as they worked in battery recycling industry (OEx) and 50 were not occupationally exposed (NOEx). Routine biochemical parameters were analyzed for screening using Modular P biochemistry auto-analyzer (Roche diagnostics, USA). *GSTT1 and GSTM1* gene polymorphisms were studied using multiplex polymerase chain reaction with *CYP1A1* as internal control. Blood lead levels (BLL) were determined using microwave digestion followed by estimation by inductively coupled plasma optical emission spectrometry (ICP-OES). Data was analyzed statistically using the GraphPad prism 5.0 software.

Results:

The distribution of InDel polymorphisms did not reveal any significant differences in distribution between the OEx and NOEx groups. BLL were found to have non-gaussian distribution and hence were compared between the genotypes by Mann Whitney U test. *GSTT1-* genotypes were observed to have significantly higher BLL compared to *GSTT1+* (p=0.024). However, *GSTM1* InDel polymorphisms did not reveal significant differences.

Conclusion:

Summarily, *GSTT1* null genotypes were found to be associated with significantly higher BLL especially in the occupationally exposed group.

Table: Blood Lead levels in genotypic subgroups in Occupationally lead exposed and non-occupationally exposed subjects:

	NOEx subjects (50)		OEx subjects (50)	
Genotype	n	Mean ± SD	n	Mean ± SD
GSTT1+	44	10.16 ± 11.6	39	40.55 ± 34.82
GSTT1-	06	11.35 ± 10.1	11	53.87 ± 43.00
GSTM1+	31	10.5 ± 10.23	28	43.61 ± 38.42
GSTM1-	19	10.03 ± 13.3	22	43.30 ± 35.34

B-290

Pre-analytical Contamination of Pediatric and Newborn Urine Drug Screening Samples with Tributoxyethylphosphate

H. A. Paul, H. Sadrzadeh, J. M. Boyd. *Alberta Public Laboratories, Calgary, AB, Canada*

Background: Urine drug testing of children is sometimes required to assess exposure to drugs of abuse. At our laboratory, these samples undergo untargeted screening by gas chromatography-mass spectrometry (GC-MS) with mass spectra matching to a library of over 400+ compounds. Recently, three pediatric and newborn urine samples collected in-hospital were found to contain a large peak identified by the library as tributoxyethylphosphate (TBEP). This peak was not seen in any adult samples over the same time period. We immediately suspected the TBEP could be coming from the collection method, as urine samples can be challenging to collect from this age group. Discussions with the units determined that the most common collection methods were either via a sterile urine collection bag system, after which the urine is transferred into a sterile urine collection container, or by placing sterile cotton balls in the diapers of infants, after which the urine is squeezed out into a sterile urine collection container. We investigated these collecting modes to determine whether we could identify the source of TBEP.

Methods: Sterile pediatric and newborn urine collection bags, diapers, sterile cotton balls, and a sterile urine collection container were obtained from one of the units where samples containing TBEP were collected. Washings were collected from each container as follows. The urine collection bags were washed with 10 mL clinical laboratory reagent water (CLRW). Cotton balls were placed in a sterile urine collection container and washed with 20 mL CLRW, after which the water was squeezed out into a new sterile urine collection container. For the diaper, 50 mL CLRW was poured into the diaper and a sterile syringe was used to aspirate the water back out and transfer the aspirate into a clean glass tube. Finally, the sterile urine collection container provided by the hospital was washed with 10 mL CLRW. All water samples were extracted and analyzed using an Agilent 6890 GC coupled to a 5973 Mass Selective Detector using the same protocol used for patient urine samples.

Results: Tributoxyethylphosphate was detected only in the water that was squeezed from the cotton balls. This compound was subsequently detected in two additional patient samples that were confirmed to be collected by cotton balls. Recently, we were made aware that the supplier of sterile cotton balls to the hospitals in our city had switched approximately 3 months previously. Repeating the cotton ball extraction using samples from both the previous and current sterile cotton balls confirmed that TBEP was only in cotton balls from the new supplier.

Conclusion: Using GC-MS analysis, we were able to identify the cotton balls used to collect pediatric and newborn urine samples as the source of TBEP, which has recently been detected in several urine samples in our laboratory secondary to a change in cotton ball suppliers. This study shows the importance of pre-analytical variables and their potential impact on comprehensive drug screening results.

B-292

Assessment of Mass Spectrometry-Based Urine Opioid Screening in Clinical Laboratories: How Well Does Your Assay Perform?

S. Delaney, D. Danso, L. Langman, P. Jannetto. *Mayo Clinic, Rochester, MN*

Background: Although there has been an overall decline in new opioid prescriptions, a crisis of drug misuse, abuse, and diversion continues to grow with overdose deaths increasing dramatically over the last decade. Urine drug testing (UDT) is an effective tool in monitoring patient compliance to prescribed opioid use and can also help identify aberrant behaviors. The recently published Laboratory Medicine Practice Guidelines (LMPG) recommend mass spectrometry (MS)-based screening assays as the first line UDT. While MS-based testing is considered to be the "gold standard" for UDT, it remains a laboratory-developed test which is not standardized (e.g. different test menus and detection limits) and requires extensive validation to prevent erroneous reporting. Specifically, non-prescribed or prescribed opioids, opioid metabolites, or concomitant medications can cause isobaric or chromatographic interferences—which can potentially confound the correct identification and interpretation of the UDT result. The clinical laboratory is responsible for accurate drug identification, reporting, and meaningful interpretation of results in order to identify adherence or misuse. The objective of this study was to assess the current state of MS-based urine drug screening for opioids in clinical laboratories throughout the United States.

Methods: Ten blinded urine samples were sent to three reference laboratories across the United States. A combination of authentic patient samples containing the drugs and metabolites of interest or drug-free urine samples spiked with various combinations of opioids and/or opioid metabolites to represent different clinical scenarios were used. The presence and final concentrations of the analytes was verified using a semi-quantitative drug screen and quantitative MS-based confirmatory assays. Assessment was based upon: 1) correct identification of compounds, and 2) appropriate interpretation of UDT results when interpretation was provided by the performing laboratory.

Results: For the identification of analytes, 85% of drugs were correctly identified and appropriately reported by the participating laboratories, while 15% of the tests failed to detect the drugs despite having concentrations above the stated cut-offs. Out of the total number of opioids included in the samples, 11% were not offered as part of the test menu. Interpretation of the results was similar with 83% of samples providing accurate interpretation of the urine drug screen including identification of possible spiked samples. However, 10% of samples were reported as compliant when the actual sample was adulterated, or had no interpretation due to a failure in detecting one of the analytes. The remaining 7% of samples were considered "not applicable" due to unavailability of the analyte in a test menu.

Conclusion: The lack of harmonization of MS-based UDT adds another layer of complexity in an already challenging environment that clinicians face when trying to correctly interpret opioid UDTs. The results from this study show that while the majority of the drugs were detected, 1 in 10 UDT would have been released with incorrect interpretations which could impact patient care. Standardization of MS-based UDT test menus, detection limits, and interpretation protocols is essential to improving patient safety by providing clear, actionable results to the clinical team.

B-293

Performance Evaluation of Drugs of Abuse Assays on the Atellica CH 930 Analyzer

A. Wagner, A. Pietz, A. Trevizo, E. Carbonneau, D. Grenache. *TriCore Reference Laboratories, Albuquerque, NM*

Background: Drug of abuse screening is performed for several purposes, including for pre-employment screening and to assist in emergency situations. The purpose of this study was to evaluate the analytical precision of specific drug of abuse screening tests performed on the Atellica CH (Siemens Healthcare Diagnostics).

Methods: The drugs of abuse screening panel on the Atellica CH utilizes Syva® *EMIT*® technology to qualitatively determine the presence or absence of drug based on a specified cut-off. Precision and concordance to Syva EMIT assays performed on a Beckman AU 680 analyzer (Beckman Coulter) was performed. Assays included amphetamines (cut-off 500 ng/mL), barbiturates (cut-off of 200 ng/mL), benzodiazepines (cut-off 200 ng/mL), cocaine (cut-off of 150 ng/mL), methadone (cut-off 300 ng/mL), opiates (cut-off of 300 ng/mL), phencyclidine (cut-off of 25 ng/mL), and cannabinoids (cut-off 50 ng/mL). Testing was performed using the qualitative mode for each drug of abuse assay. For precision, two levels of Bio-Rad Liquichek™ Qualitative Urine Toxicology Quality Control (one negative and one positive) were tested in five replicates once each day for three days. For the concordance studies, 20 samples which had tested negative for any drugs by the Beckman AU method, and 20 samples which had previously tested positive by the Beckman AU method were tested for their respective drug assays on the Atellica CH Analyzer.

Results: The precision runs recovered as expected (negative or positive) for each assay over the 3 days. Concordance between the Atellica CH drug of abuse assays and the Beckman AU drug of abuse assays was 100%.

Conclusion: The drug of abuse assays on the Atellica CH Analyzer demonstrated excellent precision and concordance to the Beckman AU drug of abuse assays.

B-294

Assessment of an Immunoassay and LC-MS/MS for the Detection of Fentanyl in Human Urine

L. Chen, A. J. Jenkins. *Quest Diagnostics, Marlborough, MA*

Introduction: Fentanyl is a synthetic opioid prescribed to treat moderate to severe pain. In recent years the illicit use of fentanyl has increased with reports of heroin, cocaine and marijuana containing fentanyl. As a result, there has been increased interest by health care providers to detect use of this drug in individuals in treatment for substance use disorder and pain management. **Objective:** To evaluate the ARK™ Fentanyl homogeneous enzyme immunoassay (ARK Diagnostics, Inc., Fremont, CA) as a screening tool for fentanyl/norfentanyl and to assess the alignment with a liquid chromatography tandem mass spectrometry (LC-MS/MS) assay. **Methods:**

Random urine specimens collected between April 2018 and January 2019 were assayed by the ARK Fentanyl immunoassay on a Beckman Coulter AU 5800 series chemistry analyzer. The instrument was calibrated daily using a 0.5 ng/mL calibrator with quality control material at 0.25 and 0.75 ng/mL. All presumptive positive screening results were assayed for fentanyl and norfentanyl by LC-MS/MS using a Prelude LC (Thermo Fisher Scientific, Waltham, MA) and SCIEX 6500 triple quadrupole MS (SCIEX, Framingham, MA). Chromatographic separation was achieved with Kinetex Biphenyl 50 x 3.0 mm 2.6 um 100A column (Phenomenex, Torrance, CA) and a gradient time program with binary mobile phase of 0.1 % formic acid in deionized water and 0.1% formic acid in methanol. Nine calibrators in the concentration range of 0.2-1000 ng/mL (Cerilliant Corporation, Round Rock, TX) produced a quadratic curve fit with a 1/x weighting. Quality control material included drug free urine and positive controls at 0.625, 400 and 800 ng/mL (Lipomed Inc., Cambridge, MA). The following transitions were monitored: fentanyl (337.2/188.2; 337.2/216.1); norfentanyl (233.2/150.2; 233.1/94.1); d5-fentanyl (342.2/188.2; 342.2/105.0); and d5-norfentanyl (238.1/155.2; 238.1/84.1). The precision of the immunoassay (IA) was determined by assaying reference material at 50 % and 150% cutoff with 5 replicates over 5 days; carryover measured by assaying low, then high followed by low concentrations over 3 runs; and sensitivity and specificity by comparing results of 82 patient specimens. **Results:** The precision of IA around the cutoff demonstrated a within run coefficient of variation (CV) of 16.7% at 0.25 and 6.5% at 0.75 ng/mL. The IA was challenged with approximately 125 potentially interfering substances at a concentration of 1,000 ng/mL. The following drugs produce positive results: 3-Methylfentanyl, 4-ANPP, acrylfentanyl, alfentanil, butyryl fentanyl, carfentanil, cyclopropyl fentanyl, furanyl fentanyl, methamphetamine, and sufentanil. No carry over was observed at fentanyl concentrations 2000 times the cutoff. The sensitivity was 100% and specificity was 84%. 1011 patient urine specimens tested presumptive positive by IA and 583 (57.7%) were confirmed positive for fentanyl and /or norfentanyl using a lower reporting limit of 0.5 ng/mL. 3 samples were reported as interference by LC/MS/MS due to ion ratio failure. In these 583 confirmed positive samples, ten (1.7%) specimens contained fentanyl only, 156 (26.6%) contained norfentanyl only, and 417 (71.2%) contained both analytes. **Conclusion:** The ARK Fentanyl IA is a sensitive but less specific screening tool for determining exposure to fentanyl.

B-295

Performance Evaluation of Therapeutic Drug on the Atellica CH 930 Analyzer

A. Wagner, A. Pietz, A. Trevizo, E. Carbonneau, D. Grenache. *TriCore Reference Laboratories, Albuquerque, NM*

Background: Therapeutic drug use is monitored by clinical laboratories to ensure medication compliance, titrate appropriate dosages, and prevent toxicity. The therapeutic drugs on the Atellica CH analyzer (Siemens Healthcare Diagnostics) are based on either the assay principles utilized on the Dimension Vista, Dimension EXL, or ADVIA Chemistry 1800 analyzers (Siemens Healthcare Diagnostics). The objective of this study was to evaluate how serum assays for carbamazepine, digoxin, lithium, phenytoin, and vancomycin on the Atellica CH Analyzer compared to the same methods performed on the Dimension EXL and/or Dimension Vista.

Methods: Residual serum samples used for clinical therapeutic drug monitoring were tested on the Atellica CH analyzer. Deming regression analysis was performed using Microsoft Excel with Analyze-It. The Validate TDM1 Calibration Verification/Linearity material from LGC Maine Standards was used for linearity testing of carbamazepine, phenytoin, and vancomycin. The Validate GC1 Calibration Verification/Linearity material from LGC Maine Standards was used for linearity testing of lithium. Linear regression analysis was used to evaluate linearity.

Results: Method comparison results are shown in the table. Carbamazepine, phenytoin, vancomycin and lithium were linear across the 5 standards tested with slopes that ranged from 0.96 to 1.04 and y-intercepts that ranged from 0.025 to 0.29 and covered ~90% of assay measuring ranges.

Assay (Units)	Measuring Interval: Dimension ExL (E), Dimension Vista (V), Atellica CH (A)	Range Tested: Dimension ExL (E), Dimension Vista (V), Atellica CH (A)	N	EXL Comparison			Vista Comparison		
Carbamazepine (ug/mL)	E: 0.0 to 20	E: 0.6 to 17.2	39	Intercept	Slope	r	Intercept	Slope	r
	V: 0.5 to 20	V: 0.6 to 17.2		0.107	0.941	0.992	-0.321	0.965	0.992
	A: 0.4 to 20	A: 0.4 to 16.0							
Digoxin (ng/mL)	E: 0.2 to 5	E: 0.21 to 3.85	40	0.002	0.828	0.995	-0.081	0.846	0.996
	V: 0.06 to 5	V: 0.28 to 3.8							
	A: 0.14 to 5	A: 0.15 to 3.17							
Lithium (mmol/L)	E: 0.2 to 5	E: 0.30 to 1.76	40	0.009	1.017	0.993	0.001	1.046	0.996
	V:0.2 to 3	V: 0.27 to 1.71							
	A: 0.1 to 3	A: 0.28 to 1.78							
Vancomycin (ug/mL)	E: 0.0 to 50	E: 4.2 to 23.4	40	0.086	0.909	0.956	-1.648	0.902	0.934
	V: 0.8 to 50	V: 4.0 to 24.3							
	A: 3 to 50	A: 4.5 to 22.8							
Phenytoin (ug/mL)	E: 0.5 to 40	E: 1.6 to 37.4	38	0.269	1.008	0.996	0.918	0.998	0.989
	V: 0.4 to 40	V: 3 to 39.9							
	A: 2 to 40	A: 2.5 to 38.0							

Conclusions: The Atellica CH TDM assays demonstrated acceptable linearity and agreement to the Dimension Vista and EXL TDM assays. The observed slope of <0.9 for the digoxin assay is attributed to differences in assay principles between the methods.

B-296

Pain Management Urine Drug Screening Using a LC-MS/MS Method for Patients Admitted through Emergency Department

S. Feng, T. Le, J. Gardiner, P. Wang, M. C. Milone, L. M. Shaw. *Hospital of the University of Pennsylvania, PHILADELPHIA, PA*

Background: In the last two decades, the increasing incidence of opioid use disorders and overdose deaths involving opioids have reached epidemic proportions in the United States. Meanwhile, the number of adults filling benzodiazepine prescriptions increased from 8.1 million to 13.5 million between 1996 and 2013, and overdose deaths involving benzodiazepines increased from 1135 in 1999 to 8791 through 2015. Emergency departments (ED) play an essential role in fighting drug overdoses. Hospital of the University of Pennsylvania toxicology laboratory started Pain Management testing in July 2007, using a combination of GC/MS and immunoassay, then expanded opioid testing with LC-MSMS in 2013. In 2018, the current LC-MS/MS method containing 43 analytes was initiated. The present study, a retrospective assessment of drug prevalence in ED patients for whom urine immunoassay-based drug screening was ordered, was conducted in order to understand clinician needs and further improve testing and reporting.

Methods: We have collected 200 consecutive urine specimens from ED patients whose urine was tested by immunoassay drug screening. Those specimens have been run both by immunoassay and LC-MS/MS.

Analytes monitored include: drugs of abuse (DOA) (THC, benzoylecgonin, amphetamine, MDA, MDMA, methamphetamine, phentermine, phencyclidine, 6-MAM, methadone, and EDDP), benzodiazepines (7-aminoclonazepam, alprazolam, alpha-hydroxyalprazolam, chloradiazepoxide, clobazam, diazepam, nordiazepam, estazolam, desalkylflurazepam, 2-hydroxyethylflurazepam, alpha-hydroxytriazolam, lorazepam, midazolam, alpha-hydroxymidazolam, oxazepam, and temazepam), and opioids (buprenorphine, norbuprenorphine, codeine, fentanyl, norfentanyl, hydrocodone,

dihydrocodeine, hydromorphone, morphine, naloxone, naltrexone, oxycodone, oxymorphone, tapentadol, N-desmethyltapentadol, tramadol, and N-desmethyltramadol). LC-MS/MS was performed on an ABSciex 3200 QTrap incorporating a Shimadzu liquid chromatographic. And the immunoassay was performed on Beckman AU5822 or Olympus AU400.

Results: ED specimens were tested on LC-MS/MS and Immunoassay. In the DOA group, THC has the highest prevalence, 28.9%. Cocaine metabolite, benzoylecgonine has a positivity rate of 11.8%. Several commonly seen benzodiazepines include lorazepam (9.2%), oxazepam (3.9%), alprazolam (2.6%), and clonazepam (2.6%). Notably, there is a high inconsistency between immunoassay and LC-MS/MS for detecting benzodiazepine. 73% benzodiazepine positive results by LC-MS/MS were negative by immunoassay, likely due to lack of a pre-analytical hydrolysis step as well as the variable sensitivity toward different benzodiazepines. Furthermore, in the opioids group, morphine has the highest prevalence, 10.5%. The 6-MAM positivity rate is only 1.3%, probably due to rapid metabolism. Oxymorphone is the second most prevalent drug with a positivity rate of 7.9%. It is not surprising that fentanyl and norfentanyl are the third most prevalent opioids with a positivity rate of 6.6%. Among those patients, one had a prescribed fentanyl patch. 40% of fentanyl positive patients were also positive for cocaine.

Conclusion: Our investigation revealed that THC has the highest positive rate in ED patients for whom urine drug screening was ordered, indicating cannabinoids are the most commonly used drug in the DOA group. Morphine is associated with the highest positivity rate in opioids. Fentanyl (6%) is also detected in ED patients. Furthermore, the high inconsistency between immunoassay and LC-MS/MS for detecting benzodiazepine due to the low and variable sensitivity of the former suggests that physicians should be cautious when interpreting results from immunoassay screening followed by reflex confirmatory testing.

B-297

Five-Year Trends in Drug Use: Urine Drug Screening Positivity Rates for Community-Based Patients in Ontario, Canada from 2014 to 2018

A. S. Ptolemy[1], H. Li[1], D. Bailey[1], P. Catomeris[2]. *[1]Dynacare, London, ON, Canada, [2]Dynacare, Brampton, ON, Canada*

Background: Comprehensive multi-year reports detailing the prevalence and annual trends in drug use within a specific patient cohort are often not widely available or current. Urine drug screening positivity rates derived from qualitative liquid chromatography tandem mass spectrometry-based (LC-MS/MS) patient testing may be used to obtain this information. This approach to identifying recent drug use trends in community-based patients in Ontario, Canada has not yet been published.

Objectives: Identify multi-year trends in drug use by examining qualitative LC-MS/MS urine drug screening positivity rates.

Methods: All LC-MS/MS urine drug screening results from 2014 (N=136,864), 2015 (N=153,329), 2016 (N=106,687), 2017 (N=75,774) and 2018 (N=71,361) were retrospectively reviewed. Following enzymatic hydrolysis and protein precipitation, all urine specimens received targeted LC-MS/MS screening which identified the presence of drugs within the following drug classes: anesthetic; anticonvulsant; antidepressant; benzodiazepine; cannabinoid; opioid; stimulant; and illicit. Relevant drug metabolites and related compounds were also included in this test. A total of N=63 different compounds were screened using their respective positive/negative cut-off concentrations. The positivity rates for all analytes were tabulated and partitioned by month of testing.

Results: Over the examined five-year testing period, urine drug screening positivity rates ranged from 76.6% (cotinine) to <0.01% (7-aminoflunitrazepam, benzylpiperazine, desalkylflurazepam, diazepam, flunitrazepam, flurazepam, JWH018, JWH200, MDEA, MDPV, mephedrone, phenazepam and triazolam). From 2014 to 2018, annual significant ($p \leq 0.05$) increases in urine drug screening positivity rates were observed for: amphetamine (3.4% to 7.7%); and gabapentin (5.4% to 8.2%). From 2015 to 2018, annual significant positivity rate increases were observed for fentanyl (3.4% to 4.9%). From 2016 to 2018, significant positivity rate changes were observed for: buprenorphine (8.7% to 11.7%); diphenhydramine (10.5% to 12.0%); levamisole (8.0 to 3.3%); naloxone (8.5% to 11.9%); and norbuprenorphine (10.0% to 13.0%). From 2017 to 2018, significant positivity rate changes were observed for: 6-acetylmorphine (1.4% to 1.0%); benzoylecgonine (13.3% to 12.2%); bupropion (2.3% to 3.0%); cotinine (72.3% to 69.5%); EDDP (37.6% to 33.2%); hydromorphone (10.0% to 8.6%); lorazepam (5.0% to 4.6%); methadone (37.1% to 33.2%); naltrexone (0.3% to 0.7%); norcodeine (5.9% to 5.4%); norfentanyl (6.8% to 7.6%); noroxycodone (15.9% to 14.9%); oxazepam (6.0% to 5.6%); oxycodone (13.5% to 12.8%); ritalinic acid (2.8% to 2.6%); and THCA (28.6% to 27.5%).Relative to the 2018 observed positivity rates, all other analytes included in the LC-MS/MS screening panel did not show significant annual trends or differences within the tested patient population.

Conclusion: This retrospective review of qualitative LC-MS/MS urine drug screening positivity rates from 2014 to 2018 identified several significant annual drug use changes. Amphetamine and fentanyl use significantly increased in 2018 while cocaine and heroin use decreased. Since 2016, the use of Suboxone has significantly increased but evidence of methadone-based opioid antagonist therapy remains more common. Oxycodone and hydromorphone use decreased significantly in 2018. Laboratories can provide detailed information on drug use trends within a specific patient population by tabulating, interpreting and communicating urine drug screening positivity rates to their clinical communities.

B-298

Preliminary Performance of the ARK™ High Sensitivity Opiates Assay on the Beckman Coulter® AU680

K. Chung, M. Abney, B. Moon, J. Valdez. *ARK Diagnostics, Inc., Fremont, CA*

Background: Three separate homogeneous immunoassays are commercially available for the detection of Morphine/Codeine, Hydrocodone/Hydromorphone and Oxycodone/Oxymorphone. A new High Sensitivity (HS) Opiates Assay for human urine screening has been developed by ARK Diagnostics, Inc. The ARK Assay is designed to detect Morphine/Codeine, Hydrocodone/Hydromorphone and Oxycodone/Oxymorphone in a single assay when calibrated with dihydrocodeine at a cutoff level of 300 ng/mL. The benefit provided by this assay is the consolidation of three assays into one, thereby minimizing cost, labor, and eliminating the need for multiple channels on the analyzer. The assay consists of ready-to-use liquid reagents that provide qualitative and semiquantitative results. The data presented in this study was generated on the Beckman Coulter AU680 System. **Methods:** Precision was evaluated by histogram overlap analysis at the cutoff and ±25% controls. Analytical recovery was studied by spiking dihydrocodeine into human urine at levels that span the assay range (50-4000 ng/mL). Fifty negative and 44 opiates-positive specimens from a pain clinic were analyzed and the results compared to those of LC-MS/MS. Cross-reactivity with structurally related drugs was assessed. **Results:** Qualitative determination of the ±25% controls showed no overlap with the cutoff by histogram overlap analysis. The assay quantified negative urine spiked with dihydrocodeine between 100-3000 ng/mL within ±10% of nominal values. The concentrations producing a signal equivalent to the 300 ng/mL dihydrocodeine cutoff ranged from 40 ng/mL for hydrocodone, hydromorphone, oxycodone, and oxymorphone to 300 ng/mL for morphine and codeine. The assay demonstrated no clinically significant crossreactivity to structurally related opioids such as naloxone, nalorphine, naltrexone, and dextromethorphan. Method comparison with LC-MS/MS showed 98% agreement: specificity 100% (50/50); sensitivity 95% (42/44). The two discordant samples showed morphine present at a concentration below 50 ng/mL. **Conclusion:** The ARK HS Opiate Assay on the Beckman Coulter AU680 System is a sensitive opiates screening method for urine specimens at a cutoff level of 300 ng/mL, providing qualitative and semiquantitative analysis of morphine, codeine, hydrocodone, hydromorphone, oxycodone, and oxymorphone in a single assay.

B-299

Comparison of Performance Characteristics between 2 Qualitative Urine Benzodiazepine Assays: Traditional versus High-Sensitivity

K. Morel, J. Chesser, A. J. McShane. *Cleveland Clinic Foundation, Cleveland, OH*

Background: Traditionally, benzodiazepine immunoassays, a common component of urine drug screens (UDS), have lacked sensitivity due to their low cross-reactivity to benzodiazepine glucuronide metabolites. To mitigate this analytical challenge, we converted from a traditional [Kinetics Interaction of Microparticles in Solution (KIMS), Integra 800, Roche Diagnostics] to a high-sensitivity assay [High-Sensitivity Cloned Enzyme Donor Immunoassay (HS-CEDIA, Thermo Scientific), c502 (Roche Diagnostics)]. The HS-CEDIA assay includes a β-glucuronidase enzyme, which removes the glucuronide molecule, and increases the cross-reactivity of benzodiazepine glucuronide metabolites. To determine if enzyme hydrolysis increased our UDS benzodiazepine sensitivity (i.e. true positive rate), a retrospective analysis was performed to determine both assay's performance characteristics.

Method: Data was collected for ±7 months after converting benzodiazepine screening assays. Only samples with a benzodiazepine screening and confirmatory testing were included in the analysis. Both screening tests, KIMS and HS-CEDIA, utilized a

threshold of 200 ng/mL. Quantitative confirmation testing was performed on a liquid chromatography tandem mass spectrome

try (LC-MS/MS) platform, and includes the following analytes: 7-aminoclonazepam, α-hydroxyalprazolam, α-hydroxytriazolam, oxazepam, lorazepam, nordiazepam, and temazepam. The lower LoQ was 40 ng/mL for all components except α-hydroxyalprazolam, which was 60 ng/mL.

Results: For the KIMS assay, 76 samples had both benzodiazepine screening and confirmatory testing performed. Of the 76 samples tested, 20 were true positives, 2 were false positives, 24 were true negatives, and 30 were false negatives. For the HS-CEDIA assay, 67 samples were included in the data. Of the 67 samples, 31 were true positives, 1 was a false positive, 27 were true negatives, and 8 were false negatives. The performance characteristics were then calculated (Table-1).

Conclusion: The HS-CEDIA assay has nearly twice the sensitivity of the KIMS assay. The increased sensitivity significantly increased the diagnostic accuracy of our urine benzodiazepine screening.

Table 1: Performance Characteristics of the KIMS and HS-CEDIA Benzodiazepine Screening Assays

	KIMS	HS-CEDIA
N	76	67
Sensitivity	40%	79%
Specificity	92%	96%
Positive Predictive Value	91%	97%
Negative Predictive Value	44%	77%
Diagnostic Accuracy	58%	87%

B-300

The Evaluation of LCMSMS Method Selectivity for Quantitative Analysis of Drugs and Metabolites

S. Wu[1], F. Hassounah[1], J. Albaghdadi[1], C. Dou[2]. [1]*Elite Medical Laboratories, Lawrenceville, GA,* [2]*Kroll Care Lab, Woodstock, GA*

Background: The separation and detection selectivities are important quality parameters for liquid chromatography-mass spectrometry based analytical method. The main factors that influence the selectivity are biological sample matrix effects and isobaric mass transitions for our toxicology drug confirmation and quantitative analysis methods. It is necessary to assess the ability of discriminating the target drugs or their metabolites from the interfering compounds during method development and validation.

Methods: Liquid chromatography mass spectrometry method (AB Sciex 4500 LC-MSMS system and a separation column of biphenyl 50 x 4.6mm 2.6um) was set up for quantitative analysis of 140 drugs and metabolites in human urine samples. Liquid chromatography mobile phase A was water with 0.1% formic acid and mobile phase B was methanol with 0.1% formic acid and operated in gradient elution. Samples were prepared through enzymatic hydrolysis and dilute and shoot procedure. Instrument parameters include chromatography separation, run time, mobile phases compositions, gradient and flow rate, and mass spectrometry parameters for multiple reaction monitoring (MRM) transitions. These parameters were optimized to achieve best separation and sensitivity. The evaluation of separation and detection selectivities were performed on the following selected analytes groups: (1) Drugs or metabolites with Isobaric mass transitions; (2) opioid group; (3) Benzodiazepines group; (4) Amphetamine family drugs and (5) Antidepressants and psychotics. Chromatograms were reviewed. Retention and separation factors and resolution were calculated for separation selectivity. For matrix effect experiments, the mass spectrometry signal responses between neat and spiked biological urine samples were compared at concentration of cutoff level.

Results: Retention factors for all analytes fell in the range of 2 to 15. The separation factors and resolutions for the analytes of isobaric mass group were 1.2 and 2.1 for morphine and hydromorphone, 1.2 and 2.1 for codeine and hydromorphone, 1.2 and 2.1 for Naloxone and 6-MAM, 1.1 and 2.4 for methamphetamine and phentermine, and 1.2 and 2.5 for desmethylvenlafaxine and tramadol, respectively. For the detection selectivity experiment, approximately 1% of spiked samples showed mass spectrometry response signals for both analyte and internal standard (IS) decreased 3 to 5 times by comparing to neat samples. However, the ratios of analyte to IS intensity were within 5% differences from neat samples for all analytes. At cutoff levels, the ratios of mass spectrometry detection signals of analytes to the background noises were still significantly greater than 10 for those samples which their signal responses decreased due to the ion suppression matrix effects. The water dilution of samples improved the signal to noise ratio.

Conclusion: Identifying separation and detection interferences, optimizing and evaluating method selectivities are necessary steps during method development and validation. These will prevent false positive or negative results for drug confirmation and quantitative analysis in a toxicology laboratory.

B-301

Assay for Select Antipsychotics Drugs By LCMSMS in Human Serum

A. Dermartirosian, E. Shahbol, K. Thomassian, A. Shah, S. Rezaei. *quest Diagnostics, valencia, CA*

Background: Antipsychotic drugs can increase the risk of sudden cardiac death, with some studies showing a threefold increase among patients treated with antipsychotics. Detection of these drugs can help physicians determine the proper treatment as well as monitor patients for these risks. Nevertheless, a high-throughput LC-MS/MS assay to test these antipsychotics was not available.

Objective: To establish and validate a definitive analysis of 18 antipsychotic analytes and metabolites in human serum by LC-MS/MS.

Method: The analytes and metabolites of interest were split into 3 groups based on their reportable range though individual LC-MS/MS analyses. Group A consists of haloperidol, trifluoperazine, fluphenazine, and (Z)-thiothixene. Group B consists of risperidone, 9-OH-risperidone, molindone, olanzapine, loxapine, 8-hydroxy-loxapine, and ziprasidone. Group C consists of chlorpromazine, aripiprazole, clozapine, norclozapine, quetiapine, thioridazine, and mesoridazine. We utilized a 96-well Cerex plate in which each well contained 200 µL of a blank, calibrator, quality control, negative, or patient sample. Then 400 µL of 5% formic acid and 25 µL of deuterated internal standards were added to each well. The plate was mixed and placed into a Cerex ALDIII Automated Liquid dispensing instrument, where the columns were washed with 0.7 mL of 2% formic acid followed by 0.8 mL of methanol. The columns were dried for 2 minutes under pressure and eluted with 300 µL of elution reagent (98% methanol + 2% ammonium hydroxide) and evaporated under nitrogen at room temperature. Then 400 µL of mobile phase A (0.1% formic acid in water) was added to the dried residue and vortexed for 1.5 min at 1,400 rpm. The samples were then ready to be analyzed by LC-MS/MS. The LC-MS/MS was equilibrated for 20 min and had 20 blank injections with retention times evaluated before starting the patient sampling. The injection volume for the samples was 15 µL. The validation process involved verifying the analytical measurement range (AMR) through linearity and recovery and determining the precision, accuracy, interference, and cross-reactivity for each analyte.

Results: An 8-point calibration curve was generated for each analyte and showed consistent linearity and reproducibility in the range of ±20% of their target with regression coefficient (r) >0.990. The Precision for each analyte fell within a TEa/3 or 10%. All quality controls were analyzed against approximately 150 drugs with no interfering substances found. The analytical measurement range (AMR) for group A was 1 to 40 ng/mL with a cutoff of 2 ng/mL. The AMR for group B was 1 to 200 ng/mL, except for loxapine (5 ng/mL), 8-hydroxy-loxapine, (5 ng/mL), and ziprasidone (10 ng/mL); the cutoff was 2 ng/mL. The AMR for group C was 20 to 1,000 ng/mL, except for thioridazine and mesoridazine (both 50 ng/mL); the cutoff was 25.

Conclusions: We developed and validated a robust definitive LC-MS/MS analysis of 18 antipsychotic drugs and select metabolites. The simple procedure reported here should be useful to clinicians for monitoring these medications and their metabolites.

B-302

Development and Validation of an LCMSMS Assay for Therapeutic Drugs in Urine

K. Thomassian, A. Dermartirosian, S. Rezaei, A. Shah, E. Shahbol. *Quest Diagnostics/Valencia, Valencia, CA*

Background: The CDC and other professional associations recommend drug testing to provide a baseline for drug-use information, as well as for ongoing monitoring throughout prescription drug therapy. Current drug tests only search for specific analytes; therefore drugs that are not being analyzed will not be detected. A high throughput method that detects a wide range of prescription, licit, and illicit drugs would allow a wider scope of screening for providers.

Objective: We developed and validated a LC-MS/MS definitive analysis for 13 therapeutic drugs and their metabolites.

Methods: This method tests for buspirone, 6-hydroxy buspirone, hydroxyzine, diphenhydramine, oxcarbazepine, 10-hydroxycarbazepine, topiramate, ketamine, norketamine, chlorpromazine, methocarbamol, cyclobenzaprine, pseudoephedrine, and zaleplon**.** In a 96 well plate 25 µL of urine samples, calibrators, and 3 levels of in-house made quality controls were spiked with 25 µL of internal standard (IS) The IS was composed of a mixture containing the deuterated analog of each analyte. The mix was diluted by adding 450 µL of sample diluent containing 75% mobile phase A (2 mM ammonium formate 99.995+% in DI H_20 with 0.1% formic acid) and 25% mobile phase B (mobile Phase B contains a 75:25 methanol::acetonitrile solution) to each well. The plate was then covered and sealed with ultra-thin Teflon adhesive film and vortexed at 1,100 rpm for 5 minutes. The plate was moved to the LC-MS/MS (Model #API 4500), which was equilibrated for 20 minutes and primed with 20 injections of blanks before running patient samples; 10 µL of sample was injected. The validation process involved verifying the linearity and recovery along with determining the precision, interference, stability, analytical measurement range (AMR), and lower limit of detection (LOD) for each analyte. This validation used standard laboratory procedures specified under CLIA regulations.

Results: A 5-point calibration curve exhibited consistent linearity and reproducibility in the range of ±20% of their target with regression coefficient (r) >0.990. The CVs were between 7.5% and 10%. The precision for each analyte fell within a TEa/3 or 10%. All quality controls were analyzed against approximately 150 drugs with no interfering substances found. For most drugs, AMR was 12.5 to 500 ng/mL and the lower limit of detection (LOD) was 12.5 ng/mL. For ketamine, norketamine, and topiramate, AMR was 25 to 1,000 ng/mL, and LOD was 25 ng/mL. For 10,11-dihydro-20-hydroxy carbamazepine and 10,11-dihydro-20-hydroxy oxcarbazepine, AMR was 50 to 2,000 ng/mL and LOD was 50 ng/mL. For methocarbamol AMR was 125 to 5,000 ng/mL and LOD was 125 ng/mL

Conclusions: We developed and validated a sensitive and robust LC-MS/MS method for detection of a wide-array of parent compounds and their selected metabolites in urine. The method demonstrates the potential for high-throughput patient monitoring for people with drug substance use disorder. The simple procedure reported here is suitable for a high-throughput clinical laboratory.

B-303

The Thermo Scientific CEDIA EDDP Immunoassay Can be Hijacked to Detect Kratom in Urine Drug Screens

C. Gineste, L. Bazydlo. *University of Virginia, Charlottesville, VA*

Background: Kratom is a plant-derived product that has recently gained popularity as an alternative to opioid analgesics in the United States. However, given recent declarations by the Food and Drug Administration (FDA), it is also becoming controversial from a regulatory standpoint. The active compound in kratom, mitragynine, is an alkaloid with opioid agonist properties. Cases of kratom toxicity are rare and often lack confirmatory analysis. Many hospital labs do not currently have a screen for kratom in their Urine Drug Screen (UDS) panel. It was observed that a patient admitted to the ER for kratom toxicity had a false positive EDDP screen using the Thermo Scientific CEDIA Methadone Metabolite (EDDP) immunoassay on the Olympus AU480 Chemistry Analyzer**.** EDDP is the major metabolite of the opioid methadone. The patient's methadone screen was negative.

Method: Reference standards for the most abundant active components of kratom, mitrogynine and 7-hydroxymitrogynine, were purchased from Cerilliant and used to make standards from 10ng/ml to 10,000ng/ml in blank urine. An LCMSMS assay was developed using the Waters Xevo microTQS Mass Spectrometer with Acquity UPLC. Data from UDS screens for all available urine specimens was monitored and samples that fell into the following groups went on to LCMSMS testing for mitrogynine and 7-hydroxymitrogynine; EDDP positive and methadone negative or EDDP indeterminate and methadone negative.

Results: An LCMSMS assay was developed with a linear range of 10-10,000ng/ml mitrogynine and 7-hydoxymitrogynine. Review of previous EDDP positive and methadone negative results identified six additional possible cases; four of these confirmed positive for methadone use, two were tested and found to be positive for kratom use. Six specimens in the indeterminate range were also identified. Analysis by LCMSMS showed that five of these six specimens were positive for mitrogynine and 7-hydroxymitrogynine. Monitoring and testing of positive and indeterminate EDDP/negative methadone UDS is ongoing to enable estimation of the sensitivity of the EDDP UDS for detection of mitrogynine.

A spiking experiment with 10,000ng/ml of mitrogynine and 7-hydroxymitrogynine resulted in an EDDP screen that was in the indeterminate range suggesting that the cross reactivity could be due to a different component or metabolite of kratom.

Conclusion: A component or metabolite of kratom found in the urine of kratom users cross reacts with the Thermo Scientific CEDIA EDDP immunoassay. We report for the first time this immunoassay being used successfully to identify patients taking kratom.

B-304

The Effect of Sample Handling on Free Valproic Acid Levels: Is It Better to Measure or Estimate Free Drug Levels?

D. Wang, E. Champion-Lyons, N. Bohm, R. Neyens, N. Babic. *Medical University of South Carolina, Charleston, SC*

Background: Valproic acid (VPA) is a broad-spectrum anticonvulsant drug. Under normal conditions, this drug is highly protein bound (85-90%). However, in patients with hypoalbuminemia, the free fraction can increase substantially while the total VPA levels remain in therapeutic range. The neurologic activity and toxicity of the drug, however, are directly related to the levels of free drug. It is, therefore, important to accurately measure or estimate levels of free VPA in these patients. The aim of this study was to: a) evaluate the variability in free VPA measurements between different laboratories and b) asses the accuracy of the equation used by pharmacists to estimate free VPA levels, based on patient albumin levels.

Methods: This study included 20 left-over, de-identified adult patient specimens with measurable free and total VPA. Both hypoalbuminemic and normoalbuminemic patients were included. VPA was measured on Abbott ARCHITECT i2000 instrument (Abbott, Abbott Park, IL) and on Roche Cobas (Roche Diagnostics, Indianapolis, IN) used by our reference laboratory (RL). To separate free drug, Centrifree (MilliporeSigma, Burlington, MA) filtration units were used. Briefly, 500 µL of patient sample was pipetted into a Centrifree filtration unit and centrifuged at 3200 RPM for 10 minutes. To assess the effect of transport conditions on free VPA levels, specimens were assayed in our clinical laboratory, immediately aliquoted and shipped to RL refrigerated and frozen. To assess the methodology bias, 20 specimens were obtained from the RL and analyzed.

Results: Analysis of samples obtained from the RL (n=20), demonstrated no clinically significant method bias between MUSC (y) and RL (x) in total and free VPA measurement. Passing-Bablok correlations were y=0.99x-3.77, r=0.995 for total VPA and y=0.90x-0.1, r=0.992 for free VPA. Comparison of patient specimens collected in our institution and sent frozen to RL confirmed a good correlation between the two methodologies for total VPA levels (y=1.03x-4.86, r=0.984). However, comparison of free VPA levels revealed significant negative bias (y=0.78x-3.86, r=0.954). The negative bias is further exaggerated if specimens were shipped under refrigerated conditions. Finally, the free drug fractions obtained from both assays showed positive bias compared to the predicted values from the equation, 5.3% (95% LoA -21.26% to 31.86%) for the in-house method and 19.43% (95% LoA -8.11% to 46.97%) for the reference method.

Conclusion: Our results indicate that, while there is no clinically significant bias between MUSC and RL for total VPA methods, significant bias is observed when levels of free drugs were compared. Furthermore, the estimated free VPA levels, based on patient albumin concentration, correlate better than RL with levels measured at MUSC. It appears that specimen shipping and handling conditions are significant contributors to this bias. This is very important since, based on recent CAP survey participant data, it is evident that majority of the labs send out free VPA. While more than 3000 laboratories perform total VPA, only 41 measure free VPA levels. Our next step, therefore, is to investigate the mechanism of sample handling effects on the VPA-protein binding kinetics.

B-305

Moving Away from Immunoassays: Adapting LC-MS/MS for Routine Urine Toxicology Screens

A. Badea, K. L. Lynch, A. H. B. Wu. *UCSF, San Francisco, CA*

Background: Immunoassays dominate the drug screen assay market as the most widely used method for routine urine toxicology screens. They have been optimized to be fast, sensitive, and easily automated. The downside is the lack of specificity, coupled with cross-reactivity from irrelevant, similarly structured compounds. Furthermore, not all immunoassays are sensitive to drug metabolites, leading to false negatives. These limitations lead to the need for confirmation testing by LC-MS/MS, which has longer turnaround times and oftentimes needs to be sent out to reference labs, or additional investigations that delay clinical decision making and this negatively impact patient outcome. The ToxTyper™ is an automated LC-MSn coupled with a method-specific library search engine and compound identification algorithm. Its push-button, user-friendly interface is promising for implementation in the clinic

without the need for toxicology trained staff. This study aims to optimize and compare the ToxTyper drugs-of-abuse (DOA) LC-MSn method with the currently used immunoassays (CEDIA and DRI) for urine toxicology routine panels in terms of number of compounds identified, speed, and sample preparation.

Methods: Analysis of remnant clinical samples was approved by the UCSF Institutional Review Board. 72 urine samples from emergency department patients were spiked with Fentanyl-d5 as internal standard and diluted 1:5; chromatographic separation of analytes was performed with an Acclaim RSLC 120 UHPLC column (C18, 2.2 um, 2.1 x 100 mm); elutes were ionized by Ion Booster ESI and data was acquired on an Ion Trap MS/MS (Bruker) using alternating positive- and negative-ion mode. The separation method and corresponding library targeted 86 common drugs of abuse and their metabolites. The ToxTyper-provided LC gradient was custom-modified to meet turnaround time requirements. Library search, compound identification, and report generation were performed automatically. The results were compared with those previously generated for these samples by CEDIA, EMIT, and DRI immunoassays (opiates, benzodiazepines, cocaine metabolite, amphetamines, oxycodone, EDDP, MAM6).

Results: Of the 72 samples, 44% were found to be negative by both methods. In 33% cases, the ToxTyper found additional drugs (8) that were not included in the immunoassay screening panel, but that could provide guidance for clinical action. In 34% of the cases, the ToxTyper provided additional information by speciating the individual opiates, benzodiazepines, or amphetamines. In 27% of the cases the ToxTyper detected drugs found under the set immunoassay cutoff, which may provide useful information for patients with mixed toxidromes, such as those in the emergency department. Sample preparation was streamlined to 1-2 minutes/sample, individual results were generated within 12 minutes, and a workflow that prevented sample-to-sample carryover allowed for the analysis of 60 samples per day, which well exceeds our daily demands. The interface was user-friendly, and sample submission did not necessitate familiarity with mass spectrometry.

Conclusion: This study demonstrated that the ToxTyper provided consistent results with commonly used immunoassay screens, within a competitive time-frame. Furthermore, the added information and specificity provided by the LC-MS/MS system can be useful in optimizing patient care. The platform is easy to use and provides a push-button LC-MS/MS alternative for urine toxicology screens.

B-306

Rhabdomyolysis: Implications of Drugs of Abuse in the Pathology

P. E. Young, P. McCaffrey, D. Djouodo-Nemzou, A. Okorodudu. *University of Texas Medical Branch, Galveston, TX*

Background: Rhabdomyolysis is a condition characterized by breakdown of skeletal muscle with release of its contents into plasma. It was first reported in English literature during World War II among Britons who were victims of a bombing campaign. Since then, rhabdomyolysis has been broadly associated with trauma and crush injuries. There is evidence however, that crush injuries may not be the cause of most cases of rhabdomyolysis.

Methods: We completed a retrospective review of patients' charts for a six-month period presenting to our hospital with creatine kinase (CK) values greater than or equal to 1000 U/L. We used this cutoff, as this is the level of CK generally accepted for the cutoff of rhabdomyolysis as described by Gabow et al, and adopted by many researchers since.

Results: 186 patients met our inclusion criteria. Of these, 20 patients did not have relevant information in their electronic medical records or the records could not be accessed, thus were eliminated. Analysis of the remaining 166 patients with CK $\geq$ 1000 revealed that drugs of abuse were associated with 32 cases (19%). Among the drugs, amphetamines were the most commonly associated (n=21), followed by cocaine (n=16), PCP (n=4), heroin (n=1). Some patients were taking multiple drugs at the same time. Another 15 cases were suspected to be drug related with documented history of drugs use in previous hospital admissions. However, these patients were not tested for drugs of abuse for various reasons. Their presenting complaints were however presumed to be drug related and treated as such. In one case, a patient was brought to the ED with altered mental status by her friends who said that they had all been using MDMA and alcohol the night before. Falls accounted for 17 cases (10%), especially in the elderly. Intense workout/exercise 16 (9.6 %), dehydration 12 (7 %), sepsis/septic shock 12 (7 %), trauma/crush injury 10 (6 %), polymyositis/dermatomyositis 8 (5 %), seizures 6 (4%), cardiogenic shock 5 (3 %), statin associated myopathy 4 (2%), neuroleptic malignant syndrome 2 (1%), were the next most commonly associated.

Conclusion: Contrary to popular belief, the major cause of rhabdomyolysis may not be crush injuries. Of all the cases we observed, drugs of abuse were associated with the majority of cases of rhabdomyolysis. This was even more significant in patients who were under the age of 50. This is in keeping with other research work which have identified drugs of abuse as the leading cause of rhabdomyolysis.

B-307

Therapeutic Drug Monitoring: Determination of Cefepime in Plasma Using High-Performance Liquid Chromatography with UV Detection

P. H. Tang. *Cincinnati Children's Hospital Medical Center, Cincinnati, OH*

Background: Cefepime is a fourth generation cephalosporin antibiotic. It has a broad spectrum of activity against Gram-positive and Gram-negative bacteria over other commercially available cephalosporin drugs. Cefepime has approximately 20 years of medical use with confirmed effectiveness in various indications. However, ensuring effective and safe drug dosing in critically ill patients can be difficult. Cefepime has been associated with a greater risk of mortality than other β-lactams in patients treated for severe sepsis. In a recent review assessing cefepime monotherapy for febrile neutropenia, the authors found increased mortality rates. Hypotheses for this failure include unrecognized toxicity or inadequate pharmacokinetic/pharmacodynamics parameters for antimicrobial efficacy. Thus, therapeutic drug monitoring (TDM) may be useful to ensure therapeutic drug levels and avoid toxicity.While analytical interference during cefepime therapeutic drug monitoring has recently reported, this might bias cefepime determination in clinical samples. Therefore, the objective of the present study was to develop a rapid and accurate method for the determination of cefepime in plasma.

Methods: Venous blood was collected into heparin evacuated tubes. Specimens were centrifuged, and plasma was transferred to amber tubes and frozen immediately. Aliquots (100 µL) of thawed and well-mixed plasma samples were used for the determination of cefepime. Measurement of cefepime in plasma was then made after deproteinization. Proteins were removed from plasma by precipitation using 100% methanol and the resulting supernatant was transferred into a vial. An aliquot of the supernatant was injected to the high-performance liquid chromatography (HPLC) system equipped with UV detection for cefepime analysis. Separation was achieved by a linear gradient elution on a reversed-phase C18 column (4.6 x 250 mm) connected to an automated HPLC system with 50 mM phosphate buffer (pH 4.6) and methanol as eluent. Cefepime and internal standard were separated from impurities in plasma. Cefepime and internal standard were detected at 260 and 244 nm, respectively and quantified by measuring peak heights.

Results: Due to the lability nature of cefepime, significant plasma- and temperature-dependent degradation of cefepime was observed. The HPLC method was validated for linearity, limit of quantification, accuracy, precision, and specificity. Measurements of cefepime were linear from 1 to 300 µg/mL with a lower detection limit of 0.3 µg/mL. Accuracy was greater than 95% of the nominal concentrations of cefepime spiked in pooled plasma samples. Imprecision was <10%. The absence of matrix interferences was achieved. The method allows measurement of cefepime in plasma in 11 min.

Conclusion: The method was proved to be suitable for real-time monitoring of cefepime concentrations in plasma.

B-308

A Validated, Rapid Method for Detecting a Broad Panel of Pain-Management Drugs in Oral Fluid by High-Resolution LC-MS/MS

A. Badea, A. H. B. Wu, K. L. Lynch. *UCSF, San Francisco, CA*

Background: Increased prescription and use of pain-management drugs has led to an observed increased risk of misuse and addiction, making drug monitoring of patients essential in preventing undesired outcomes. While urine is traditionally the preferred specimen for drug testing due to its noninvasive sample collection, large sample volume, and longer detection window relative to blood, it still presents some shortcomings such as ease of adulteration, uncomfortable supervised collection, and unavailability in the case of anuric patients. Oral fluid is emerging as a promising biological matrix for drug testing due to its noninvasive collection and minimal potential for adulteration. We have developed a high resolution mass spectrometry method for detecting a panel of 82 pain-management drugs and metabolites in oral fluid, compatible with the widely used Quantisal® oral fluid collection device.

Methods: Oral fluid samples were collected with the Quantisal® device. For limit of detection studies, Quantisal® negative synthetic saliva pre-diluted in storage buffer was spiked with standard for each drug. The sample preparation was performed by

in-tip solid-phase extraction (SPE, DPX®) and automated via a ViaFlo® electronic 96-channel pipette. Chromatographic separation of analytes was performed using a Kinetex C18 column with a 10-minute gradient from 2%-100% organic. Data was collected on a SCIEX TripleTOF®5600 using a positive-ion mode TOF-MS survey scan with IDA-triggered collection of high resolution product ion spectra (20 dependent scans). Limits of detection and matrix effects were determined for 86 pain management drugs and illicits/drugs of abuse. LOD was defined as the lowest concentration for which the drug met scoring criteria for positive identification in duplicate injections and had a signal-to-noise ratio >20:1. Matrix effects were determined by calculating the mean signal intensity in urine (N=5 matrix samples, tested in triplicate) minus the mean signal intensity in water (N=3 water samples, tested in triplicate) divided by the mean signal intensity in water and multiplied by 100%.

Results: The drug panel studied consists of 82 opiates, opioids, synthetic opioids, benzodiazepines, stimulants, SSRIs, SNRIs, anti-psychotics, muscle relaxants, sedatives, and illicits. LODs are as follows: 33% of the drugs were detected at a concentration of 1 ng/mL, 23% at 5 ng/mL, 11% at 10 ng/mL, 8.5% at 25 ng/mL, 8.5% at 50 ng/mL, and 16% at 100 ng/mL or higher. Matrix effects ranged from -90% to 655%, with most (89% of analytes) falling betwen -61% and 41%. The method was validated in 39 patient oral fluid samples and yields results comparable to more focused oral fluids panels documented in literature and compatible with the SAMHSA advised screen cutoffs, where available.

Conclusion: We have developed a high resolution mass spectrometry method for the detection of 82 pain- management/illicit drugs and metabolites in oral fluid. The sample preparation is fast, automated, and yields LODs consistent with previously documented methods for more focused drug panels. This method is the first to target such a wide and diverse panel of drugs using this matrix, and is suitable for use in clinical labs as a complement or replacement of urine drug testing methods.

B-309

Implementation of Lamotrigine and Levetiracetam TDM by ARK Diagnostics, Inc. Immunoassays

K. T. Calayag[1], J. Yee[2], D. Zhou[2], R. Shi[1]. *[1]Stanford University School of Medicine, Dept. of Pathology, Palo Alto, CA, [2]Stanford Health Care, Clinical Chemistry, Palo Alto, CA*

Introduction: Lamotrigine (Lamictal®) and Levetiracetam (Keppra®) are oral anticonvulsant and anti-epileptic drugs used to treat seizures in patients with epilepsy and bipolar I disorder. Therapeutic drug monitoring is recommended to optimize dosage and to determine patient compliance. Conventionally, Lamotrigine and Levetiracetam levels are determined by using LC-MS/MS which can be a time-consuming process that requires manual sample preparation. We implemented and validated user-defined immunoassays on an automated chemistry analyzer using FDA approved test kits from ARK Diagnostics, Inc.

Methods: ARK Lamotrigine and Levetiracetam assays are homogenous enzyme immunoassays for quantitative determination of Lamotrigine and Levetiracetam in human serum or plasma designed for clinical chemistry analyzers. Parameters for both assays were determined and optimized for Siemens Vista. They were validated for clinical diagnostic use and their performance was assessed to include precision, analytical recovery, linearity, limit of quantitation, and accuracy by correlation studies.

Results: Lamotrigine Assay: Intraday (n=10) CV%s were 7, 4, 6 for concentrations of 2, 12, 25 µ/mL; interday (n=20) CV%s were 7, 11, 13, respectively. Analytical recovery ranged from 90 - 132%. The AMR was (0.85 - 40 µg/mL), linearity was confirmed: y = 0.96x + 0.4 (0 - 40 µg/mL, R^2=1.0). Correlation study result of HPLC(x) vs ARK(y) using Passing-Bablok regression analysis was: y = 1.06x + 0.001 (n=30; R^2 = 0.99). Levetiracetam assay: Intraday (n=10) CV%s were 4, 4, 5, for concentrations of 7, 30, 75 µ/mL; interday (n=20) CV%s were 9, 9, 8, respectively. Analytical recovery ranged from 108 - 128%. The AMR was (2 - 100 µg/mL), linearity was confirmed: y = 0.95x + 1.02 (5 - 100 µg/mL, R^2=0.99). Correlation study result of HPLC(x) vs ARK(y) using Passing-Bablok regression analysis was: y = 1.13x - 0.29 (n=29; R^2 = 0.99).

Conclusion: The ARK Lamotrigine and Levetiracetam immunoassays on the Siemens Vista platform demonstrated satisfactory performances with strong correlations to results determined by LC-MS/MS and have the benefit of efficiency with rapid turn-around time and with no manual sample preparation.

B-310

Lead Testing in A New Mexico Population: A Comparison of Screening and Follow-Up Testing Patterns

D. E. Sosnovske[1], M. Dodd[2], D. Grenache[2]. *[1]University of New Mexico, Albuquerque, NM, [2]TriCore Reference Laboratories, Albuquerque, NM*

Background: Environmental exposure to lead is a preventable threat to the optimal health and development of children. The American Academy of Pediatrics (AAP) supports a focused screening for lead exposure in high-risk pediatric populations. A common screening sequence is to test whole blood lead from a capillary sample and confirm result >5 ug/dL with a venous sample. The aim of this study was to evaluate lead testing patterns in locations served by TriCore Reference Laboratories and determine appropriateness of testing based on AAP guidelines.

Methods: Lead test results from September 2013 to November 2018 were obtained from the laboratory data warehouse. If an individual was tested more than once, the earliest test was considered the initial test and the latest test was considered a follow-up test. Intervening tests were not assessed. Test results were categorized into not detectable (<5 ug/dL), elevated (>=5 and <10 ug/dL) and very elevated (>=10 ug/dL). Lead testing was performed by inductively coupled plasma mass spectrometry.

Results: A total of 12,920 lead tests were resulted over the five year period. Sample types included capillary whole blood on filter paper (22%), capillary whole blood (1%), and venous whole blood (77%). There were 80 elevated venous samples, 27 (34%) of which received repeat venous whole blood testing. There were 44 very elevated venous samples, 26 (59%) of which received repeat venous whole blood testing. There were three elevated capillary samples, two (66%) of which did not have repeat testing and one (33%) that was repeated with venous whole blood testing. Of 53 elevated filter paper samples, 25 (47%) had no additional testing, five (9%) were re-tested with filter paper testing, and 23 (43%) were re-tested with a venous whole blood sample. There were 15 very elevated filter paper samples, four (27%) of which did not have repeat testing, one (7%) that was repeated with a filter paper sample, and 10 (67%) that were followed by venous whole blood testing.

Conclusion: Primary lead testing in New Mexico is most commonly performed with venous blood rather than by capillary blood. Patients with elevated or very elevated blood lead concentrations were often, but not always, re-tested. Repeat testing for abnormal capillary blood lead concentrations were not always appropriate. For example, filter paper samples were repeated with a second filter paper sample when venous whole blood is the recommended confirmatory test.

Wednesday, August 7, 2019

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

Technology/Design Development

B-311

Establishment of a Highly Sensitive COLD-PCR/FMCA Method for Simultaneous Quantitative Detection of HBV DNA, Genotype and RT Mutation and Its Application for Chronic Hepatitis B Diagnosis

C. Liu, Q. Ou, J. Lin, X. Zhen. *The department of medical laboratory science, Fuzhou, Fujian Province, China*

Background: Dynamic and real-time HBV DNA, genotype and RT mutation analysis plays an important role in diagnosing and monitoring chronic hepatitis B (CHB) as well as in assessing the therapeutic response. We aimed to establish a highly sensitive coamplification at lower denaturation temperature PCR coupled with probe-based fluorescence melting curve analysis (COLD-PCR/FMCA) for precision diagnosis of CHB patients.

Methods: The amplification at lower denaturation temperature and FMCA were combined to establish a COLD-PCR/FMCA assay. The performance of the method was evaluated and the results were compared with qPCR, Sanger sequencing and next generation sequencing, respectively. The samples from 650 HBV chronic infection patients (eAg-positive infection, I, n=150; eAg-positive hepatitis, II, n=150; eAg-negative infection, III, n=200 and eAg-negative hepatitis, IV, n=150) and 41 CHB patients treated with ETV were assayed by COLD-PCR/FMCA.

Results: The precision with %CV and detection limit of HBV DNA detected by COLD-PCR/FMCA were 2.58%~4.42% and 500 IU/ml. For mutation, the precision and detection limit were 3.35%~6.49% and 1%. Compared with Sanger sequencing, the coincidence rates of genotype and mutation were 96.0% and 82.5%, whereas the inconsistent data was caused by a low proportion (<20%) of mixed genotypes or mixed mutations. The mutation ratio in HBV infection patients was shown as: I (0/0.0%)<III (16/4.5%)<II (30/5.5%)<IV (36/6.5%). In patients with ETV therapy, the proportion of mutation at baseline or week 4 in virological response (VR) group was < 4%, whereas in partial VR group was mostly ≥ 4%.

Conclusion: COLD-PCR/FMCA provides a novel tool with high sensitivity, convenience, and practicability for the simultaneous quantification of HBV DNA, genotype, and mutation. It might be used for distinguishing the different phases of HBV infection and predicting VR of CHB patients.

B-312

Highly Specific Enrichment of Rare Nucleic Acids Using *Thermus Thermophilus* Argonaute

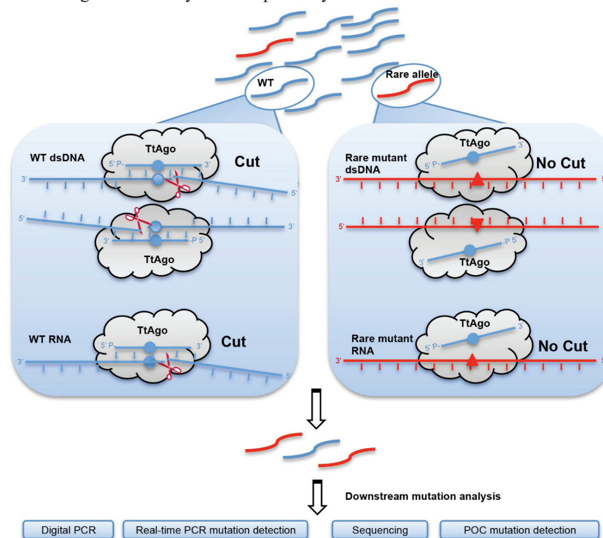
J. Song[1], J. Hegge[2], J. v. Oost[2], H. Bau[1]. [1]*University of Pennsylvania, Philadelphia, PA,* [2]*Wageningen University, Wageningen, Netherlands*

Background: Characterization of disease-associated, cell-free nucleic acids (liquid biopsy) provides a powerful, minimally-invasive means for early disease detection, genotyping, and personalized therapy. Detection of alleles of clinical interest is often challenged by their low concentration and sequence homology with the much more abundant wildtype nucleic acids.

Methods: Ago from the thermophilic bacterium *Thermus thermophilus* (*Tt*Ago) utilizes short DNA guides to specifically cleave complementary DNA and RNA targets. We found that under optimized conditions, *Tt*Ago cleaves DNA and RNA complementary to the guide DNA with high efficiency, but spares nucleic acids with a single nucleotide mismatch at and around its catalytic site with high sensitivity. Based on these findings, we designed a new multiplexed enrichment assay, dubbed NAVIGATER (**N**ucleic **A**cid enrichment **Vi**a DNA **G**uided **A**rgonaute from ***T**hermus th**er**mophilus*), that utilizes *Tt*Ago, to specifically cleave perfectly complementary DNA and RNA while sparing alleles of interest.

Results: NAVIGATER greatly increases the fractions of rare alleles with single nucleotide precision enhancing the sensitivity of downstream detection methods such as ddPCR, sequencing, and clamped enzymatic amplification. We demonstrate *60-fold* enrichment of *KRAS* G12D in blood samples from pancreatic cancer patients and over ten-fold improved sensitivity of clamped-PCR (PNA and XNA-PCR), enabling multiplex detection of *KRAS and EGFR mutants* at 0.01% fractions.

Conclusion: *Tt*Ago has important advantages over enrichment assays such as the ones based on CRISPR-Cas. It does not require the target to contain a protospacer-adjacent motif; it is a true (turnover) catalyst; it can cleave both DNA and associated exosomal RNA targets, improving sensitivity; and can operate at elevated temperatures for higher selectivity and compatibility with detection schemes.



B-313

Manual Kinetic Determination of Blood Glucose in Emergency and Intensive Care Laboratories by Adapting a Commonly Used Glucose-Oxidase Reagent

R. G. M. Menendez. *Hospital Universitario Faustino Perez, Matanzas, Cuba*

There are two general approaches to enzymatic substrate analysis: (a) an end-point or equilibrium analysis, and (b) rate or kinetic measurement analysis. Taking into account the technological advances in the current photometers now disposables for manual colorimetric procedures in many small laboratories serving emergency or intensive care departments, mainly their facilities for kinetic studies, we evaluated the feasibility of a rate approach by adapting a commercially available glucose-oxidase reagent commonly used for end-point glucose determination and originally proposed by Trinder since the sixteen years of the past century. The rate method was adapted in three different photometric analyzers: Microlab 200 from Vital Scientific NV (Spankeren, The Netherland) and the models AE-600 and AE 600 N from Erma Inc. (Tokyo, Japan). Both the Microlab and the Erma models visualize a curve graph of absorbance vs. time when a kinetic test is programmed that let us choose the most suitable delay and measuring times in order to get the best results. By processing serum or plasma samples (n = 97) three regression equations were obtained: Y = 1,07 X - 0,17 (r = 0,998; p <0,001); Y = 1,04 X + 0,08 (r = 0,999; p <0,001) and Y = 1,18 X + 0,03 (r = 0,999; p <0,001) for Microlab, Erma AE-600 and Erma AE 600 N respectively when kinetic results (y) were compared with equilibrium results (x) for the same samples. The within-run and between-run precision was good and similar for both methods in the three instruments as well as their sensitivity to interferences. The rate method showed some important advantages: 1- Not requires reagent blank, 2- Calibration with a reference solution is not necessary for each run and 3-It does not need an incubation time of at least 5 minutes at 37 degree Celsius which is very important for equilibrium analysis. Therefore, the manual rate glucose analysis of one or a few samples per run as it is demanded in the emergency and intensive care laboratories during 24 hours daily is much more rapid and less expensive than the end-point analysis with similar levels of accuracy and precision.

B-314

Circulating Liver Specific miR-122 qPCR Assay as a Potential Biomarker of Drug Induced Liver Injury for Small Molecule Compounds

m. kibukawa, L. Chen, M. J. Marton, O. F. Laterza, D. Levitan. *Merck, Kenilworth, NJ*

Background: Small molecule compounds used in the discovery and development of new drugs may cause potential liver toxicity after high dose administration. To evaluate the safe administration of clinically relevant doses of a compound, a miR-122 RT-qPCR assay was developed and validated as an early and more sensitive alternative biomarker of liver toxicity. miR-122 is expressed specifically and abundantly in liver tissue and it has been shown that its expression level in the liver and plasma concentration increase after drug-induced injury. This is correlated with increased level of plasma alanine aminotransferase (ALT) activity, which is the current standard for assessing drug induced liver injury. However, increases in ALT activity can also result from muscle disease or activity. To clarify the cause of elevated ALT activity, the expression level of miR-133, a muscle-specific miRNA associated with muscle activity, was also monitored. To ensure consistent performance of miRNA extraction from plasma samples, ath-miR-159a, a plant specific microRNA, was assessed from Arabidopsis RNA spiked into plasma samples in order to normalize for recovery rate. We report on the analytical validation of these three microRNA assays, including assay linear dynamic range, analytical precision, inter-subject biological variance, and analyte stability.

Methods: MicroRNA was extracted from plasma samples by using miRCURY RNA Isolation kit-biofluids (Exiqon). However, since this isolation kit is no longer commercially available, we also used the miRNeasy Serum/Plasma Advanced Kit (Qiagen) after finding it as an acceptable substitute. Arabidopsis total RNA that contains miR159a was spiked-in with MS2 RNA carrier into the plasma samples to normalize for recovery differences. Performance of microRNA extraction was investigated with 103 healthy donor plasma samples to assess whether this normalization method was suitable to calculate miRNA copy number. Validation experiments for these miRNA assays were performed using 40 healthy donor plasma samples and 45 patient plasma samples with type 2 diabetes to determine the assay linear dynamic range, analytical precision, inter-subject biological variance, and analyte stability. Synthetic-mature miRNA 122, 133a and 159a were purchased from Sigma and used to generate a Synthetic-mature RNA titration curve for calculation of miRNA copy number per 1ml plasma.

Results: (1) %CV of recovery rate was 17.7% and met acceptance criteria for normalization of microRNA expression level in plasma. (2) The assay can reliably (<30%RSD) detect miRNAs copy number down to 199 copies per qPCR reaction for all three miRNA assays. (3) Intra-run and inter-run repeatability of end-to-end analytical performance for two target miRNA assay was <30% and met acceptance criteria. (4) miRNA in plasma samples are stable at -80C for up to three years. (5) Significant effect of hemolysis on the ability to detect levels of miR-122 and miR-133a in plasma was not observed.

Conclusions: Based on these results, the microRNA extraction protocol, TaqMan miRNA assays, and normalization method are considered fit-for-purpose validated. Plasma specimens from clinical studies of small molecule compound administration in healthy donors may be used as an exploratory biomarker to test the hypothesis that miR-122 plasma concentration is associated with liver injury.

B-315

Plasma Betaine, a Gut Microbiome Related Metabolite, is Associated with Incident Type 2 Diabetes Mellitus in the PREVEND Study

E. Garcia[1], M. C. J. Osté[2], E. G. Gruppen[3], D. W. Bennett[1], E. Jeyarajah[1], I. Shalaurova[1], J. D. Otvos[1], S. J. L. Bakker[2], R. P. F. Dullaart[4], M. A. Connelly[1]. *[1]Laboratory Corporation of America Holdings (LabCorp), Morrisville, NC, [2]Department of Nephrology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands, [3]Department of Nephrology and Department of Endocrinology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands, [4]Department of Endocrinology, University of Groningen and University Medical Center Groningen, Groningen, Netherlands*

Background: Lower plasma betaine concentrations have been reported in subjects with type 2 diabetes mellitus (T2DM) as well as in subjects with non-alcoholic steatohepatitis (NASH). In addition, betaine, and other gut microbiome related metabolites, such as trimethylamine-N-oxide (TMAO), choline and γ-butyrobetaine, have been shown to be associated with risk of cardiovascular (CV) events. However, few studies to date have explored the association of betaine with incident T2DM. The goals of this study were to evaluate the performance of a newly developed betaine assay and explore the potential clinical associations of betaine and future T2DM in a large prospective cohort of apparently healthy adults.

Methods: We developed a novel, high-throughput, nuclear magnetic resonance spectroscopy (NMR)-based procedure for acquiring spectra that allows for the accurate quantification of circulating betaine and TMAO. Assay performance for betaine quantification was assessed and Cox proportional hazards regression was employed to evaluate the association of betaine with incident T2DM in 4,336 participants in the Prevention of Renal and Vascular Endstage Disease (PREVEND) study (mean age 52.6 ±11.5 years).

Results: The LOB, LOD and LOQ for the betaine assay were 6.4, 8.9 and 13.2 μM, respectively. Betaine assay results were linear (y= 1.02X – 3.75) over a wide range of concentrations (26.0 to 1,135 μM). Coefficients of variation (%CV) for intra- and inter-assay precision ranged from 1.5-4.3 and 2.5-5.5%, respectively. The reference interval, in a cohort of apparently healthy adult participants (n=5,621), was determined to be 20.5 to 62.9 μM with a mean of 38.0 ± 11.3 μM. In the PREVEND study (n=4,336; excluding subjects with baseline T2DM), higher betaine levels were associated with older age as well as with lower body mass index (BMI), total cholesterol, triglycerides and high sensitivity C-reactive protein (hsCRP). During a median follow-up of 7.3 (IQR, 5.9-7.7) years, 224 new T2DM cases were ascertained. Cox proportional hazards regression models revealed that the highest tertile of betaine was associated with a lower incidence of T2DM. The hazard ratio (HR) for the crude model was 0.61 (95% CI: 0.44-0.85, P=0.004). The association remained significant even after adjusting for multiple clinical covariates and T2DM risk factors, including fasting glucose. The HR for the fully adjusted model was 0.50 (95% CI: 0.32-0.80, P=0.003).

Conclusions: The newly developed NMR-based betaine assay exhibits performance characteristics satisfactory for its use in the clinical laboratory. Betaine levels may be useful for assessing risk of future T2DM.

B-316

A Matrix Stabilizing SAA Utilized as Calibrator or QC Material

Q. L. Yu[1], L. Shen[1], Q. H. Jin[1], C. H. Xiao[1], Y. D. Cao[2], S. B. Li[3], R. T. Tang[4], J. Ge[1]. *[1]Hunan Yonghe-sun Biotechnology Co., Ltd, Changsha, China, [2]Hunan Provincial People's Hospital, Changsha, China, [3]The People's Hospital of Liuyang, Changsha, China, [4]Hunan Aerospace Hospital, Changsha, China*

Background: Serum amyloid A (SAA) is a family of polymorphic proteins encoded by multiple genes and belongs to acute phase reaction proteins. The reference range of SAA in normal human body is less than 10 mg/L. Inflammation or some other diseases increase rapidly in the early stages to 1000 times the initial concentration and decrease rapidly in the recovery period of the disease. The sensitivity of SAA is much higher than C-reactive protein (CRP). Therefore, SAA may provide a better basis for earlier diagnosis and treatment. During our SAA assay development, we found that SAA was rapidly degraded once spiked into the regular diluent. The purpose of this study is to create and develop a stable SAA calibrator or QC material with a special matrix.

Methods: The SAA recombinant antigen was obtained from Chun-Lei Biotechnology Co. Ltd, Beijing, China. Five matrices with different formulations were prepared in-house. The samples with different concentrations of SAA antigens were spiked into each matrix, respectively. The stability study was designed as follows: (Group 1) samples with liquid format were placed at 37°C and 2-8°C, respectively; (Group 2) samples with freeze-dried format were placed at 37°C. The evaluation of SAA stability in each group was performed using the fluorescence-based immunochromatographic assay (Lu Yang Bioscience Co. Ltd, Hunan Provence, China).

Results: The SAA samples in liquid format at 37°C were found rapidly degraded on Day 1 at 1.0 mg/L (degradation rate=85%). However, the degradation rate of SAA at 1.0 mg/L with liquid format at 2-8°C was observed at 25.16% (Day 7), 29.37% (Day 11), 39.07% (Day 37), and 58.46% (Day 52), respectively. The freeze-dried SAA samples at 1.0 mg/L stored at 37°C were found considerably stable, e.g., the degradation rate was found only 0.9% (Day 52), 1.10% (Day 21) and 5.69% (Day 38). The recipe of the best matrix selected was created as follows: bovine serum 10% (Tianhang Biotechnology Co. Ltd.,Zhejiang Provence, China), PBS buffer 80%, pH=7.2, BSA 1% (Sigma), casein 1% (Sigma), ProClin 300, 0.02% (Sigma) , StabilZyme® SELECT Stabilizer, 10% (Surmodics, USA). In addition, we found that casein with optimal concentration may be an important element to reduce non-specific background.

Conclusion: In this study, we created and developed a matrix which especially enabled SAA more stable and could be used as SAA calibrator or QC material in clinical laboratories and IVD manufacturing.

B-317

The Cobas® SonicWash Reduces Sample Carryover on Cobas c 503 and Cobas ISE Analytical Unit

A. Seiler[1], U. Pauselius-Fuchs[1], C. Proebstl[1], E. Donnerbauer[1], A. von Meyer[2], R. Falbo[3], I. Brandt[4], J. Song[5], K. Klopprogge[6], S. Zimmermann[7], M. Horstmann[1], A. McCaughey[7]. [1]*Roche Diagnostics GmbH, Penzberg, Germany,* [2]*Klinikum St. Marien, Amberg, Germany,* [3]*Azienda Socio Sanitaria Territoriale di Monza, P.O di Desio, Desio, Italy,* [4]*OLV Ziekenhuis, Aalst, Belgium,* [5]*Seoul National University Bundang Hospital, Seongnam, Korea, Republic of,* [6]*Roche Diagnostics GmbH, Mannheim, Germany,* [7]*Roche Diagnostics International Ltd, Rotkreuz, Switzerland*

Background: Sample to sample carryover on diagnostic analyzers may lead to erroneous patient results. This inherent risk needs to be addressed especially for analyzers that do not apply disposable tips for sample pipetting. In particular, sample carryover may be detrimental in configurations where clinical chemistry and immunoassay analyzers are combined in integrated systems. Since the concentration of immunoassay analytes frequently differs by several logs, carryover on clinical chemistry analyzers must be prevented to avoid elevated immunoassay test result. To improve sample carryover on the new **cobas® pro** integrated solutions, a wash station with ultrasonic cleaning and basic wash solution (**cobas** SonicWash) was developed for **cobas c** 503 and **cobas** ISE analytical unit.

Methods: Sample carryover with **cobas** SonicWash was quantified by processing samples with high analyte concentrations on **cobas c** 503 or **cobas** ISE analytical unit. Subsequently, sample probes were cleaned by **cobas** SonicWash, followed by a pipetting of an analyte-free sample. To simulate potential analyte accumulation, sample pipetting was performed repeatedly (15-times) from each sample cup. Potential carryover was determined by measuring analyte concentration on a **cobas e** 801 analytical unit.

Results: To quantify carryover significantly below the design specification of <0.1 ppm, samples with high concentrations of hepatitis B surface antigen (HBsAg) or antibodies to HBsAg (Anti-HBs) were used. In addition, a monoclonal IgG antibody with biotin- and ruthenium-label (IgG-Bi-Ru), which is known for its strong adhesiveness, was applied as sample material. Carryover of IgG-Bi-Ru was found below 0.04 ppm and 0.006 ppm on **cobas c** 503 and **cobas** ISE, respectively. Tests with HBsAg and Anti-HBs showed a carryover even below 0.004 ppm and 0.0004 ppm, respectively. Finally, results from MCE performance evaluation revealed no detectable ß-hCG carryover from patient samples.

Conclusion: In summary, the new sample probe wash with ultrasound on **cobas c** 503 and **cobas** ISE showed sample to sample carryover significantly below 0.1 ppm. Carryover of the native analytes HBsAg and Anti-HBs was found even several logs below the specification of <0.1 ppm, underlining the high cleaning efficiency of **cobas** SonicWash. In conclusion, **cobas** SonicWash strongly reduces the risk of sample to sample carryover and increases flexibility for sample routing on **cobas pro** integrated solutions.

B-318

Analysis of High-Thoughput Data from Complete Blood Count: A Year-Long Study

C. T. Trujillo[1], P. S. Osorio[1], B. F. Camara[1], P. S. Elias[1], F. E. Ciamponi[2]. [1]*Diagnosticos da America (DASA), Barueri, Brazil,* [2]*State University of Campinas (UNICAMP), Campinas, Brazil*

Background: Clinical laboratory tests (CLT) provide valuable information for diagnostic, prevention or treatment of diseases and disabilities. Several factors need to be considered when releasing these exams, from the use of appropriate controls to the reliability of the results. There are two major issues when dealing with large-scale diagnostic medicine laboratories. The first is that, although each exam produces multiple results, not all of them have the same weight for the diagnostic, with some having more leniency than others for diverging from the established standardized values. Second, the number of exams which are executed per day, ranging in the tens of thousands of tests. Such volume of information is impractical for each exam to be manually curated by human professionals and approved in a timely manner to be of practical use in health facilities. These issues make large-scale CLTs a challenging and complex process. This work demonstrates the implementation of an automated system that gathers and compiles CLT results in a quarriable database, which is then processed by a decision-making algorithm that performs cross-referencing with patient history and ranks parameters based on biological relevance for medical diagnostic, then tags the exam to be released to medical professionals or if there was an issue that require further evaluation.

Methods: As proof-of-concept, one-year worth of results from complete blood count (CBC) tests (aprox. 2X10e6), performed in Sysmex XN-3100 platforms, collected from an automated batch release system were analyzed and compared the results with the same type of exam when released by a group composed of 12 human professionals during the same period. For each exam, quantitative results, system-logged timestamps, type of release (batch, technical or medical) and final status (released or repeated) were extracted. Qualitative and quantitative statistical analysis was performed in accordance to World Health Organization (WHO) standards. Gold-standard values for turnaround time (TAT) and error rate for complete blood count exams were set based on guidelines from the brazilian National Health Agency (ANS).

Results: This work shows that automated systems can handle a much higher volume of exams per day (aprox. 5x10e3) than the human counterpart (aprox. 1x10e3), with significantly shorter time required for each test. Furthermore, automated decision-making algorithms are much less prone to errors, with an average number of "mislabeled" tests being less than 2%, while human show an average error rate of 11%. Also, the system is capable of prioritizing exams which require specialized medical review based on potential health hazard, with an average queue time of 16min for high-risk exams and 83min for low-risk exams.

Conclusion: In summary, this work demonstrates that automated systems are critical for the analytical routine of large-scale diagnostic medicine laboratories. Not only they prove to be much more efficient in handling large amounts of data, they are also much less prone to producing errors. This, in turn, allows medical professionals to better focus on results which pose health risks, allowing a quicker and better response in the care of patients.

B-319

Result Precision and Reliability on Cobas Pro Integrated Solutions Demonstrated at 4 Sites in Europe and Asia

C. Schneider-Thauern[1], O. Lorenz[2], A. von Meyer[2], C. Giacobone[3], M. Bussetti[3], R. Falbo[3], P. Brambilla[3], G. van Causbroeck[4], I. Brandt[4], K. Lee[5], J. Song[5], K. Klopprogge[1]. [1]*Roche Diagnostics, Mannheim, Germany,* [2]*Klinikum St. Marien, Amberg, Germany,* [3]*Azienda Socio Sanitaria Territoriale di Monza, Desio, Italy,* [4]*OLV Ziekenhuis, Aalst, Belgium,* [5]*Seoul National University Bundang Hospital, Seongnam, Korea, Republic of*

Background: The new **cobas® pro** integrated solution system from Roche Diagnostics, includes three high throughput analytical units, two for general chemistry testing (**cobas®** ISE and **cobas® c** 503) and one for immunochemistry analysis (**cobas®** e 801). During a multicenter study at four sites, we tested the analytical performance and the overall system functionality. Here we report on the result precision and the reliability demonstrated during the ~10-week study. **Methods:** QC materials at two analyte concentration levels were measured for the applied assays (ISEs, general chemistries and specific proteins, immunochemistry assays) per site throughout the study. The analyte recovery per QC was closely monitored. Precision studies based on the guidance from CLSI EP5-A3 were conducted over 21 days for a selection of 19 applications that represent the entire assay menu. The overall system functionality was tested under simulated intended-use conditions; the experimental design supports the analysis of results generated while samples were batch-type tested versus during full randomized routine-like testing. **Results:** From the CLSI EP5-A3 experiment, 152 CVs were calculated each for repeatability and intermediate precision. Repeatability CVs (within-run): all 24 were ≤ 1% for ISE, 77 of 80 were ≤ 2% for enzymes, substrates and specific proteins on the **cobas c** 503 and 45 of 48 were ≤ 2.5 % for immune chemistries on the **cobas e** 801 analytical units. Intermediate (within-lab) precision CVs: all 24 were ≤ 2% for ISE, 71 of 80 were ≤ 2% for enzymes, substrates and specific proteins and all 48 were ≤ 3.5 % for immune chemistries. The result precision observed for 44 assays in pooled serum, urine and WB sample material during randomized testing, is only marginally higher than that during batch-type testing. 93 % of 176 random mode CVs were < 2%. CVs derived from randomized testing that exceed 2% were still well within the respective assay specification for standard within-run precision, and what clinically required.**Conclusions:** The results of this study demonstrate that the new system delivers reliable, precise results.

B-320

Comparability of Selected Assays on the Cobas Pro® Integrated Solutions under Routine Like Conditions

P. Metz[1], O. Lorenz[2], A. von Meyer[2], C. Giacobone[3], M. Bussetti[3], R. Falbo[3], P. Brambilla[3], G. van Causbroeck[4], I. Brandt[4], K. Lee[5], J. Song[5], K. Klopprogge[1]. [1]*Roche Diagnostics, Mannheim, Germany,* [2]*Klinikum St. Marien, Amberg, Germany,* [3]*Azienda Socio Sanitaria Territoriale di Monza, Desio, Italy,* [4]*OLV Ziekenhuis, Aalst, Belgium,* [5]*Seoul National University Bundang Hospital, Seongnam, Korea, Republic of*

Background: The new Roche Diagnostics serum work area laboratory analyzer **cobas® pro** integrated solutions was evaluated in a multi-center study at four sites in Belgium, Germany, Italy and the Republic of Korea from June to October 2018. Here we report on the comparability of results between **cobas pro** systems and the respective routine analyzers of the different laboratories under routine-like conditions for 18 selected analytes covering ion selective electrodes (ISE), clinical chemistry and immunochemistry. **Methods:** For electrolytes (Cl, K, Na), clinical chemistry (ALBU, ALT, AST, Ca, Chol, Crea, CRP, Gluc, HbA1c, Phos) and immunochemistry (FT4, NT-proBNP, TnT, TSH, VitB12) a total of 18 analytes were assessed on the **cobas pro** system for their comparability to the respective routine analyzers (Beckman Coulter AU5822, Roche **cobas®** 6000 and **cobas®** 8000) with routine left-over samples at 4 evaluation sites. Passing/Bablok regression analysis resulted in slopes, intercepts and correlations for method comparison analysis. **Results:** More than 20,000 result pairs were included in the analysis. All 18 assays showed good comparability between **cobas pro** systems and the initial results on the routine analyzers. A sum of 65 method comparisons (over all 4 sites, not all sites were able to include enough sample for all 18 applications) from the **cobas pro** multi-center evaluation showed a median Passing/Bablok regression slope of 1.02, a median bias at the medical decision point of -1.8% and a median Pearson's r coefficient of 0.995. **Conclusion:** This study's results show that the **cobas pro** system delivers comparable and accurate results for a selection of 18 assays at four different evaluation sites under routine-like conditions.

B-321

Validation of Newly FDA-Approved Kappa and Lambda Free Light Chain Assays on a Previously Untested Platform

P. MULUHNGWI[1], C. N. Sharp[1], N. Pozzi[2], R. J. Elin[1], S. A. Jortani[1]. [1]*University of Louisville School of Medicine, LOUISVILLE, KY,* [2]*University of Louisville Hospital, LOUISVILLE, KY*

Background: Kappa (κ) and lambda (λ) free light chains (FLCs) are monitored to aid in the diagnosis of plasma cell disorders including multiple myeloma, Waldenstrom's macroglobulinemia and AL amyloisdosis. Typically, κ and λ FLCs are measured using nephelometric or turbidimetric methods. Previously, our lab used the Freelite assay on the Roche Cobas Integra for evaluation of FLCs. Recently, Diazyme κ and λ FLC assays were approved by the FDA. **Objective:** Our goal was, for the first time, to validate the Diazyme Human κ and λ assays on Beckman Coulter UniCel DxC 800 Synchron, and compare to the Freelite κ and λ assays on Roche Cobas Integra. Addition of the FLC method to our automation line in the lab was of interest due to greater efficiency and better turn-around-time. **Method:** Analytical performance including calibration and linearity, within run precision (n=20), qualitative and quantitative (concordance analysis) method comparison and reference range (RR) validation were conducted according to CLSI guidelines (EP 5-A2, 6, 9-A2, 12). The performance characteristics goals for the Diazyme κ FLC assay included a total allowable error (TEa) of 10%, and systematic error budget (SEa) of 2.5% (25% of TEa). For the Diazyme λ FLC assay, TEa goal was 15% and SEa was 3.75%. Statistical analysis was performed using EP Evaluator® Release 12.1.0.18. Mean, SD and CV, Deming regression, bias and percent bias plots were determined where appropriate. **Results:** The Diazyme κ FLC assay was linear with an error of 1.3% within a range of 0.00-191.00 mg/L. The Diazyme λ FLC assay was linear within 0.00-205.30 mg/L, with an error of 1.8%. For Diazyme κ FLC assay: QC1 had an observed mean of 16.70 mg/L, CV of 7.0% and ±2SD was 14.36-19.04 mg/L. QC2 had an observed mean of 33.37 mg/L, CV of 2.6% and ±2SD of 31.63-35.11 mg/L. For Diazyme λ FLC assay: QC1 had an observed mean of 21.73 mg/L, CV of 2.3% and ±2SD of 20.74-22.73 mg/L. QC2 had an observed mean of 42.05 mg/L, CV of 1.5%, and ±2SD of 40.83-43.28 mg/L. Quantitative comparison of the DxC-Diazyme FLC platform to the Integra-Freelite platform showed a -2.55 mg/L bias for κ FLC, and a 4.54 mg/L bias for λ FLC. Concordance analysis of the κ FLC assay (n=32) on both platforms showed 100% agreement for both normal (within RR) and abnormal (out of RR) values. The λ FLC assay (n=28) showed 75% (6/8) agreement for normal values and 95% (19/20) agreement for abnormal values. For κ/λ ratio (n=28) 100% concordance for normal values, and 50% (9/18) concordance for abnormal values was observed. For reference range verification, 1/20 samples was outside the URL of the Diazyme κ RR. For λ, all samples were within the manufacturer's RR. For the κ/λ, 5 samples (3 Diazyme and 2 Freelite) had values outside manufacturer's suggested RR. **Conclusion:** We conclude that the Diazyme assays for both κ and λ FLCs have excellent precision, accuracy and comparability to Freelite assays. We observed a negative and positive bias for κ and λ FLC respectively between platforms.

B-322

A Hand-powered Sample Preparation System for Pathogen Diagnosis

Y. Shin, E. Lee, C. Jin, B. Koo. *Asan Medical Center/University of Ulsan College of Medicine, Seoul, Korea, Republic of*

Background: The diagnosis of pathogens is a critical issue in global healthcare, especially in resource-limited settings with little or no access to sophisticated laboratory techniques. They generally involve intricate pre-treatments (such as pre-fabrication or sample centrifugation), which require laboratory-based procedures involving multiple steps, skilled technicians, and specific instruments. These drawbacks limit the application of NAT to point-of-care testing (POCT), especially in resource-limited settings.

Methods: Here, we describe a simple, universal protocol for use in nucleic acid testing-based pathogen diagnostics, which requires only hand-powered sample preparation, including for the processes of pathogen enrichment and nucleic acid isolation. The protocol uses low-cost amine-functionalized diatomaceous earth with a 1-μm Teflon filter as a reaction matrix in both stages of the process, using homobifunctional imidoesters.

Results: Using a simple syringe as a pump, the capture efficiency for a large sample volume (<50 mL) was enhanced by up to 98.3%, and the detection limit was 1 CFU/mL, 100-fold better than that of common commercial nucleic acid isolation kits. Our proposed system is simple, low-cost, universal, and rapid (taking <20 min), and it works regardless of the ambient environment and sample pretreatment, requiring no electricity or instruments.

Conclusion: Its benefits include the simplicity of producing its components and its ease of operation, and it can easily be integrated with other assays for point-of-care diagnostics.

B-323

Evaluation of the BD Vacutainer Eclipse UltraFill Blood Collection Needle in Comparison with the BD Vacutainer Eclipse Blood Collection Needle for Sample Quality, Tube Filling Times and Subject Pain Perception

A. Mouser, S. Hasan, M. Parikh, A. Ahuja, J. Berube, A. LaBarck. *BD, Franklin Lakes, NJ*

Background: Sample collection time and sample quality are key elements for laboratory efficiency. The BD Vacutainer® Eclipse™ UltraFill™ Blood Collection Needle (Eclipse UltraFill) was developed with an ultra-thin wall cannula to provide an increased inner cannula diameter for enhanced blood flow and faster tube filling. A study measured tube filling times using Eclipse UltraFill and the BD Vacutainer Eclipse Blood Collection Needle (Eclipse). A second study evaluated each device for sample quality (i.e., hemolysis) and subject pain perception.

Methods: In study 1, tube filling time (in seconds) was calculated with a timer using 60 samples for each device. In study 2, venipunctures (using each device/21G and 22G) were performed on 99 subjects with blood collected into 5-mL SST™ and 10-mL Serum Tubes. Hemolysis-sensitive analytes (i.e., LDH and K) and Hemolysis Index (HI) were tested on the Roche cobas® 6000. Clinical equivalence was established for each analyte when the mean bias and 95% confidence intervals were within the clinical acceptance limit. Pain was assessed against a non-inferiority criterion of 1.6 relative pain units.

Results: Mean filling times (in seconds) were: Eclipse UltraFill, 21G: 6.73; Eclipse, 21G: 15.48; Eclipse UltraFill, 22G: 9.66; Eclipse, 22G: 30.41. Comparative analysis between the devices showed a 56.55% reduction in fill time (21G) and a 68.23% reduction in fill time (22G) with the Eclipse UltraFill. Clinical equivalence was demonstrated for Eclipse UltraFill compared with Eclipse for K and LDH. Eclipse UltraFill also met the criterion for HI, as it was less than 20 with 95% confidence. For pain perception, Eclipse UltraFill was non-inferior to Eclipse.

Conclusions: The increased inner cannula diameter of the Eclipse UltraFill blood collection needle facilitates faster tube filling than the Eclipse blood collection needle,

potentially reducing sample collection time and improving efficiency. No difference between the devices was observed for sample quality or pain perception.

B-324

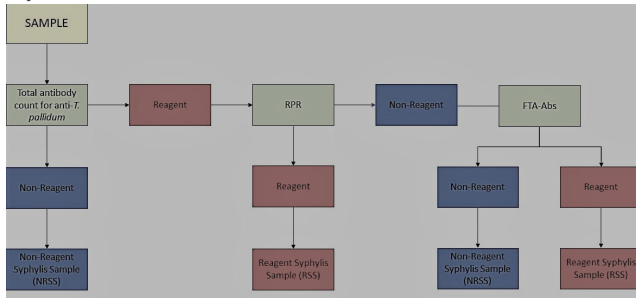
Diagnostic Automation for Syphilis

P. S. Elias[1], B. F. Camara[1], C. T. Trujillo[1], F. E. Ciamponi[2], P. S. Osorio[1].
[1]*Diagnostico da America (DASA), Barueri, Brazil,* [2]*State University of Campinas (UNICAMP), Campinas, Brazil*

Background: Syphilis is a systemic infection caused by *Treponema pallidum*, and if it is not treated early, it may develop into a chronic disease with long-term irreversible sequelae, therefore, a rapid diagnosis of syphilis is extremely important. Clinical laboratories are an important part of syphilis diagnosis, and are increasingly seeking the release of more accurate and rapid results. **Methods:** This work demonstrates the implementation of an automated system that collects, compiles, unifies and deposits the test results, following an established flow of actions, which is then processed by a decision algorithm that cross-references the results found, determining the conclusion of the examination. **Results:** The analysis starts with a treponemal test using the CMIA methodology. When the sample shows a non-reactive result, the conclusion is defined as "Non-Reagent for Syphilis Sample" (NRSS); but when inconclusive or reagent, it opens a non-treponemal test (VDRL / RPR) to confirm the diagnosis. If the results of these tests are reagent for both, the result is defined as: "Reagent for Syphilis Sample" (RSS). If there are divergences between the treponemal and non-treponemal test results, a third test with a different methodology is performed. In this case, we apply the fluorescent treponemal antibody absorption (FTA-Abs) test, which is an indirect immunofluorescence test, reducing the incidence of false-positive results (Figure 1).

If the result is reactive only in the first treponemal and non-reactive test in the other two tests, the first test is considered to be a false positive result and the result is defined as "NRSS". If the result is reagent in the two treponemal tests and nonreactive in the non-treponemal test the final result is defined as "RSS".

Conclusion: This flow greatly reduces the chance of false-positive results, thus improving test reliability and the security of the execution process during the different steps.



B-325

Analytical Performance Evaluation of 8 Assays on the Abbott Alinity ci Integrated Analyzer

Z. Mohammed-Ali[1], Y. Wang[2], P. Cheng[2], M. L. Bhandari[3], V. Kulasingam[4].
[1]*University of Toronto, Toronto, ON, Canada,* [2]*University Health Network, Toronto, ON, Canada,* [3]*Abbot Diagnostics, Abbott Park, IL,* [4]*University of Toronto, University Health Network, Toronto, ON, Canada*

Background: Various studies have characterized the analytical performance of the Abbott Alinity ci system, an integrated clinical chemistry and immunoassay testing platform. However, the performance of key analytes including 5 chemistries: Albumin (ALB), Creatine Kinase (CK), Lactate Dehydrogenase (LDH), Uric acid (UA), Hemoglobin A1c (HbA1c) and 3 immunoassays: Testosterone, Intact PTH (iPTH) and Cortisol is yet to be reported.

Objectives: To evaluate the Alinity ci integrated analyzer for imprecision, linearity, method comparison against the Abbott ARCHITECT system and Sigma metric calculation.

Methods: Five chemistries (ALB, CK, LDH, UA, and HbA1c) and three immunoassays (testosterone, iPTH and cortisol) were examined. Precision studies consisted of analyzing 2-3 levels of QC in duplicate, twice per day for 5 and/or 20 days for each analyte. Linearity across the analytical measuring range was assessed using the commercially available Main Standards Validate materials. Method comparison studies against the ARCHITECT platform was performed on a minimum of 100 patient samples (plasma/serum/whole blood depending on assay) spanning the linear range. Sigma metric calculation was determined using total allowable error goals and bias calculated against the ARCHITECT system using Passing-Bablok regression analysis.

Results: Total precision performed well with %CV ranging from 0.5 to 2.6% for chemistry assays and 1.8 - 6.1% for immunoassays across all levels. Alinity systems' assays met linearity expectations with an average recovery of 100.3%. Method comparison results showed excellent correlation between the Alinity ci and ARCHITECT platforms with linear correlation coefficients ranging between 0.99 to 1.00 and slopes of regression between 0.97 and 1.03. Mean percentage bias between 1% and 1.8% was observed. All the assays evaluated had higher than five Sigma values at or near medical decision levels.

Conclusion: Our results demonstrated excellent performance of the Alinity ci system for the 8 analytes that is comparable to the ARCHITECT platform.

B-326

Throughput and Overall Equipment Effectiveness (OEE) of Alinity ci System in a Routine Clinical Laboratory Environment

S. Promnuch[1], N. Kumproa[1], N. Teerakanjana[1], C. Chen[2], S. Vanavanan[1].
[1]*Division of Clinical Chemistry, Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand,* [2]*Scientific Services, Abbott Laboratories, Singapore, Singapore*

Background and Objective: Clinical laboratories are under pressure to produce high quality results within the quickest possible time. The objective of the study is to evaluate the operational efficiency of Abbott Alinity ci System under actual routine conditions, with focus on instrument throughput and Overall Equipment Effectiveness (OEE).

Method: Instrument operation was evaluated for a period of 5 days. In this period the Alinity ci System was used exclusively for the analysis of routine health screening specimens received during morning peak hours. Specimens were loaded onto the instrument as soon as they were received and processed preanalytically. The throughput and OEE of the instrument were evaluated. The OEE was calculated as the product of the instrument availability rate, performance rate and quality rate (refer to the equations below). As the number of the immunoassay (IA) test orders for the routine health screening specimens was not high enough to reach the full capacity of the Alinity iSystem, an additional simulation test was also carried out by using an IA panel consisting of one STAT assay and four routine 2-step assays for 90 specimens to evaluate the throughput and OEE of the Alinity iSystem at the full load. This simulation test was repeated on 2 days.

OEE = instrument availability rate (A%) x Performance rate (P%) x Quality rate (Q%), where

A% = Instrument Available Time (Instrument Time - Unscheduled Downtime)/Instrument Time

P% = (Total Production in Tests/Total Processing Time)/ Instrument throughput specification

Q% = (Total Production in Tests - Total Scrap or Exception)/Total Production in Tests

Results: An average of 200 routine health screening specimens each day were received during morning peak hours and loaded onto the instrument according to the routine lab specimen reception schedule. Out of a total of 10528 tests completed, 10261 tests (or 97%) were ordered as Chemistry assays tested on the Alinity cSystem. The average peak throughput of the Alinity cSystem was 1017 tests per hour, with a maximum peak throughput at 1125 tests per hour (Manufacturer's instrument throughput specification: 1350 tests per hour). The average OEE of the Alinity cSystem was 69.3% (A% = 100%, P% = 69.5%, Q% = 99.7%), with the maximum OEE of 77.2% (A% = 100%, P% = 77.2%, Q% = 100%). In the additional simulation test for the Alinity iSystem, the peak throughput was found to be 182 and 181 tests per hour (Manufacturer's instrument throughput specification: 200 tests per hour), with the OEE at 73.8% (A% = 100%, P% = 73.8%, Q% = 100%) and 73.6% (A% = 100%, P% = 73.8%, Q% = 99.8%), respectively, on 2 days.

Conclusion: The Alinity ci System demonstrated good throughput and Overall Equipment Effectiveness under our routine testing conditions, enabling the laboratory to achieve challenging operational performance goals.

B-327

Development of New Biochip Arrays for the Determination of Five Biomarkers Related to Acute Kidney Injury Applied to the Evidence Investigator Analyser

G. Donnellan[1], E. McCole[1], C. Adamo[2], T. Carlson[2], S. O'Donnell[1], C. Richardson[1], R. I. McConnell[3], A. A. Sethi[2], J. V. Lamont[3], S. P. FitzGerald[3]. *[1]Randox Teoranta, Dungloe, Ireland, [2]Pacific Biomarkers, Seattle, WA, [3]Randox Laboratories Ltd, Crumlin, United Kingdom*

Background: Acute Kidney Injury (AKI) is a syndrome characterized by the loss of the excretory function within a few hours of renal insult. AKI occurs in nearly 20% of all hospitalized patients and is most common in critically-ill patients with a subsequent increased risk of Chronic Kidney Disease (CKD). Increased serum creatinine and decreased urine output are used to diagnose AKI utilizing Kidney Disease: Improving Global Outcomes guidelines. However, serum creatinine is a non-specific and trailing index of AKI, therefore there is a need for more sensitive and timely biomarkers of AKI. Biochip Array Technology (BAT), by employing discrete miniaturized assays on the biochip surface, allows the determination of multiple analytes from a single sample and therefore increases the test result output and the information output per sample. The objective of this study was to develop new biochip arrays for the determination of five AKI biomarkers - clusterin, cystatin C, Kidney Injury Molecule 1 (KIM-1), Neutrophil Gelatinase-Associated Lipocalin (NGAL), and osteopontin for application in clinical test settings. The application to the dedicated benchtop semi-automated biochip analyser Evidence Investigator allows the analysis of up to 54 biochips at a time.

Methods: Chemiluminescent sandwich immunoassays were employed and applied to the Evidence Investigator analyser. Sensitivity, recovery, intra-assay and inter-assay precision, specificity, and interference were evaluated in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. A test method comparison, between the developed biochip immunoassays and commercially available individual Enzyme-Linked Immunosorbent Assays (ELISA), was completed using a cohort of 39 urine samples from normal subjects.

Results: The analytical evaluation of the immunoassays showed the following assay sensitivity values: 11.90ng/mL (clusterin, assay range: 0-1000ng/mL), 0.65ng/mL (cystatin C, assay range: 0-180ng/mL), 48pg/mL (KIM-1, assay range: 0-4000pg/mL); 0.50ng/mL (NGAL, assay range: 0-100ng/mL), and 42.60ng/mL (osteopontin, assay range: 0-8000ng/mL). Recovery of low, medium and high concentration materials was within 94.65% to 112.63% for all analytes. Average intra-assay precision and inter-assay precision values, expressed as CV (%), were, respectively, as follows: 7.96% and 8.30% (clusterin), 6.81% and 7.44% (cystatin C), 6.28% and 10% (KIM-1), 6.09% and 7.61% (NGAL), 8.83% and 9.14% (osteopontin). Cross-reactivity analysis determined that each individual assay was specific for its target, no cross-reactivity was observed with non-panel homologous proteins (cross-reactivity $<1\%$). No significant interference was found with common interferents tested. Urine samples assessment and comparison with predicate ELISAs, validated at Pacific Biomarkers, yielded correlation values of $r^2 > 0.85$ for all the assays.

Conclusion: This study indicates optimal analytical performance of five AKI related immunoassays on the biochip platform. This provides a valuable and reliable multi-analytical tool, indicating clinical utility with potential for the early and rapid detection of AKI on the Evidence Investigator platform.

B-328

Reducing the Turnaround Time of the Pre-analytical Phase by Application of a Rapid Centrifugation Profile

S. Frankenberger[1], I. Hoefer[2], B. Godber[3], A. Huisman[2], R. Stokwielder[2], M. Wesseling[2], J. den Hartog[2], J. Quint[1], S. Kasse[1]. *[1]Beckman Coulter Biomedical GmbH, Munich, Germany, [2]University Medical Center (UMC), Utrecht, Netherlands, [3]Beckman Coulter, Co. Clare, Ireland*

Background: Specimen centrifugation in clinical laboratories is one of the most time-consuming pre-analytical activities, which can take up to 15 minutes. In order to enable a higher sample throughput, we compared a centrifugation profile of 4,000g for 4 min with clinical standard profiles and investigated whether this rapid centrifugation profile is clinically acceptable for a broad panel of clinical tests. Platelet count was determined as an indicator of sample separation quality.

Methods: Blood collected from 153 healthy donors into 2 lithium heparin tubes with gel separator (BD Vacutainer® PST™ II) and 2 sodium citrate tubes (BD Vacutainer®) was centrifuged using a Beckman Coulter Centrifuge (rotor radius: 175.5 mm) at 4,000g for 4 min or with the control profile (1,200g for 10 min for lithium heparin tubes or 1,500g for 15 min for sodium citrate tubes) and was tested for 30 routine and hemolysis-sensitive clinical chemistry tests, 9 routine and fibrin-sensitive immunoassays, 4 coagulation tests and platelet count.

Blood collected from 40 healthy donors into 2 sodium citrate tubes was also centrifuged at 4,000g for 4 min or with the control profile (at 1,500g for 15 min) and then tested for platelet count and two special coagulation tests (Factor VIII and IX) using fresh and frozen-thawed samples.

Results: Weighted Deming Fit regression analysis demonstrated comparable results across the range of sample concentrations tested for each included analyte. No clinically significant difference for hemolysis-sensitive tests (AST, K, LDH) was detected nor did we find increased hemoglobin concentration. The achieved sample separation quality was high as indicated by platelet count being on average below 10,000 platelets/µl. Clinical acceptable results were obtained for Factor VIII and IX using fresh and frozen-thawed samples.

Conclusions: The data demonstrates equivalence for tests of all three disciplines between the centrifugation profile 4,000g for 4 min and the control profiles. This centrifugation profile thereby provides a solution which considerably shortens the time required for obtaining test results and which concurrently preserves high sample quality.

Abbreviations: AST = Aspartate Aminotransferase, K = Potassium, LDH = Lactate Dehydrogenase

B-329

A Novel Quantitative Multiplex Real-Time RT-PCR for the Simultaneous Detection of Enteroviruses and Major Four Subtypes

G. Xie, B. Lou, Y. Chen. *Clinical In Vitro Diagnostic Techniques of Zhejiang Province, Department of Clinical Laboratory, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China*

Background: Hand, foot and mouth disease (HFMD) is a common infectious disease caused by human enterovirus infection, with rash symptoms on the hands, feet, mouth and buttocks as the main clinical symptoms. However, serious cases and deaths have also been reported. In most of the previous reports, the pathogens causing HFMD were mainly enterovirus 71 (EV71) and Coxsackie virus A16 (CVA16). In recent years, there have been more and more epidemic cases of HFMD caused by other enterovirus subtypes other than EV71 and CVA16.Such as, CVA6 and CVA10 infections in Singapore caused 35.3% of cases of HFMD in 2008; the main pathogens of HFMD cases were CVA6 and CVA10 in France and Finland in 2010; It was made up of EV71, CVA16, CVA6 and CVA10 in 2012 in Shenzhen, which accounted for 89.8% of the enterovirus. The main pathogens of HFMD in children have changed significantly from 2013 to present in Hangzhou, China. CVA6 has become the most important pathogen, and CVA10 also has potential threats. At present, the pathogenic diagnosis of HFMD is still based on the detection of enterovirus or EV71 and CVA16 in China, which will lead to unclear or missed detection during the patient's treatment. Therefore, it's urgent to develop a method that can detect and differentiate of enterovirus and major subtypes. it's urgent to develop a method that can detect and differentiate of enterovirus and major subtypes. **Methods:** Clinical specimens, including throat swabs and stools, were obtained from the patientsby clinically diagnosed or suspected of HFMD. Total viral RNA was extracted from each specimen and specific detection of enteroviruses and major subtypes was performed using a multiplex real-time RT-PCR assay. PCR primers targeted the conserved 5' UTR gene of enteroviruses and the VP1 gene of EV71, CVA16, CVA6, and CVA10 virus. **Results:** The one-step multiplex quantitative real-time RT-PCR assay specifically detected enteroviruses and EV71, CVA16, CVA6, and CVA10 subtype, and and no cross-reaction with other enteroviruses was observed. The limitation of detection (LOD) of the multiplex assay was about 1.0×10^3copies for ten-fold gradient dilution of the positive pseudoviral particle with copied number. **Conclusion:** These results indicate that multiplex assays can simultaneously detect and differentiate enteroviruses and their major subtypes EV71, CVAl6, CVA6 and CVA10 with reliable sensitivity and specificity, which is essential for clinical diagnosis , laboratory emergency diagnosis of Enterovirus outbreak and epidemiological study of HFMD.

B-330

Comparison of Tube Filling Times Using the BD Vacutainer Eclipse UltraFill Blood Collection Needle with Currently Marketed Blood Collection Needles

A. Mouser, A. LaBarck, S. Chakravarthy, J. Hayes, S. Hasan, M. Parikh, A. Ahuja. *BD, Franklin Lakes, NJ*

Background: Improving sample collection time may help to enhance laboratory efficiency. The BD Vacutainer® Eclipse™ UltraFill™ Blood Collection Needle (Eclipse™ UltraFill™) incorporates an ultra-thin wall cannula that enables reduced tube filling time by virtue of a larger inner diameter. This study measured tube filling times using the Eclipse™ UltraFill™, 21G-22G, in comparison with currently marketed, same gauge blood collection needles (BD Multi-Sample (MSN), Eclipse™, Greiner Vacuette, Visio Plus, Kima MSN, Sarstedt Safety, Smiths Jelco, Nipro, Terumo MSN).

Methods: Tube fill time (in seconds) was measured with a timer using 60 samples of citrated sheep's blood per gauge for each blood collection needle. Mean fill time and 95% confidence interval for the mean were determined for each device. A positive mean fill time difference indicated that the Eclipse™ UltraFill™ had a faster fill time than the comparator device. Analysis of variance was performed to determine the statistical significance of this difference ($p<0.05$), as well as percent reduction in fill time, calculated as the mean fill time difference divided by the comparator group fill time. Fill speed ratio was also determined between the Eclipse™ UltraFill™ and comparator groups by dividing the mean fill time of the comparator group by the mean fill time of the Eclipse™ UltraFill™.

Results: For 21G, mean fill time differences were all positive and ranged from 5.13 - 10.10 seconds. Across all comparator groups, the Eclipse™ UltraFill™ device demonstrated a 43.25% - 60.02% reduction in fill time, correlating to 1.52x - 2.50x faster fill speed. For 22G, mean fill time differences also were all positive and ranged from 10.93 - 25.85 seconds. Across all comparator groups, the 22G Eclipse™ UltraFill™ device demonstrated a 53.08% - 70.13% reduction in fill time, correlating to 1.79x - 3.35x faster fill speed.

Conclusion: The larger inner cannula diameter of the Eclipse™ UltraFill™ facilitates faster tube filling as compared with the other marketed blood collection needles evaluated, which may help laboratory professionals improve efficiency.

B-331

Performance of Integrated Abbott Architect i2000 with Beckman Coulter PE System for Hepatitis Testing

C. Baste, D. M. Loeffler, C. Brana-Mulero, A. Shah, L. Filson, M. Jin. *University of Illinois at Chicago, Chicago, IL*

Background: Total laboratory automation (TLA) has taken over the clinical laboratory scene with an array of high throughput instruments, innovative technology, and advanced informatics. The capacity to integrate platforms across vendors and disciplines has become an essential feature in the design of TLA due to constraints of some systems, including space and test menu options. However, data on its performance is lacking. Our study objective is to evaluate the integration of a third party immunoassay platform to the TLA system for the performance of hepatitis testing using complex algorithms for the management of specimens and test results.

Methods: We use the Beckman Power Express (PE) system. Instruments connected to PE line are two Beckman AU5800, two Beckman DxI 800, and two Abbott Architect i2000, with other accessory components. The PE system is managed and interfaced to the laboratory information system (LIS) through Beckman Remisol (middleware) and Cennexus (track software). The hepatitis tests are performed on the Abbott Architect i2000 using Abbott Instrument Manager (middleware) for test resulting, interfaced with LIS and Cennexus. Using Viewics and Microsoft Excel, the monthly test volumes and TAT of hepatitis results were analyzed in two periods, pre- (2/2017-1/2018) and post- (2/2018-1/2019) integration.

Results: The average monthly TAT for each hepatitis test has decreased significantly, ranging from 42 to 129 minute reduction (p-value <0.05 for all tests) after the integration. The standard deviations of TAT are also decreased for each test, which indicates a more streamline operation (Table 1).

Table 1. Hepatitis testing TAT pre and post integration of Abbott Architect i2000s to the Beckman TLA

Test	Result / Repeat Needed	Feb 2017 - Jan 2018		Feb 2018 - Jan 2019		TAT Reduction (min)	P Value
		Volume Monthly Average	TAT (SD) Monthly Average (min)	Volume Monthly Average	TAT (SD) Monthly Average (min)		
HBCM	N, E, P / No	223	265 (51.9)	207	168 (23.2)	-98	0.00002
HBCT	N, P / Yes	390	231 (117.3)	437	169 (33.3)	-62	0.02848
HAVG	N, P / No	355	382 (172.7)	400	253 (22.8)	-129	0.01358
HAVM	N, E, P / No	272	233 (43.8)	285	154 (17.0)	-79	0.00014
HBSAB	N, E, P / Yes	853	293 (98.1)	898	211 (19.8)	-83	0.00574
HBSAG	N, P / Yes	1,308	241 (47.6)	1,382	199 (29.6)	-42	0.01083
HCVAB	N, E, P / Yes	2,515	534 (69.3)	2,276	432 (32.5)	-103	0.00015

Note: HBCM - Hepatitis B Core Antibody IgM, HBCT - Hepatitis B Core Antibody Total, HAVG - Hepatitis A Antibody IgG, HAVM - Hepatitis A Antibody IgM, HBSAB - Hepatitis B Surface Antibody, HBSAG - Hepatitis B Surface Antigen, HCVAB - Hepatitis C Antibody, N - Negative, E - Equivocal, P - Positive, SD - Standard Deviation

Conclusion: Our findings show the significant improvement of TAT of hepatitis testing with the integration of the third party Abbott Architect i2000 to Beckman PE system. In addition, the synchronization of multiple middlewares for specimen management and result reporting allows the laboratory to achieve new efficiencies handling reflex tests and managing human resources.

B-332

Comparison between Manual and Automated Cell Harvesting Processes at the Cytogenetic Laboratory - DASA Brazil

M. D. M. Silva, A. C. M. Palumbo, J. M. Fenandes. *DASA, Barueri, Brazil*

Background: With the advancement of technology and the increasing search for laboratory diagnoses, came the convenience and need of the automation of some processes, bringing with it the question of the impacts of this automation on the final quality of the tests performed.

Objective: When choosing a laboratory automation, it is necessary to think about cost of obtaining and maintaining the instrument, volume of exams to be processed and time to be saved, so that we can compare the effectiveness of the automated process to the manual.

Method: Cells harvesting to obtain the metaphases and chromosomes. This stage of the process guarantees a big part of the quality in the final result, and the manual process can present human failures. The automation of this procedure has brought advantages from an economic point of view, considering the optimization of process time, which allows the technician to perform other functions in parallel, and minimizing the waste of the low stability reagents used in the routine.

Results: When comparing productivity, a laboratory technician in a productive eight-hour work routine is trained to harvest 40 quality samples. The automation of the process in the DASA Group by the instrument CellSprint enabled us to raise this number to 96 samples. From the point of view of quality and standardization of the tests performed, the possibility of cross contamination and exchange of samples and reagents is zero, and the integrity of the material is guaranteed at the end of the step.

Conclusion: The automation of the harvesting process had advantages when considering the final quality of the tests performed, as can be seen in the images below (figure 1 - manual harvesting and figure 2 - automated harvesting). However, the volume of tests performed by the laboratory justifies the value to be invested in obtaining and maintaining the instrument.

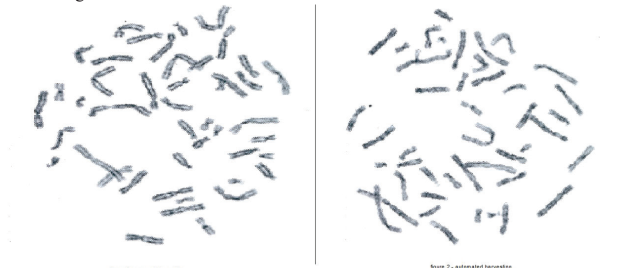


figure 1 - manual harvesting

figure 2 - automated harvesting

B-333

Evaluation of the New Multi-Test VITROS® XT Chemistry Products Slides* Assay Quality versus the CLIA TE_a Requirement

T. DiMagno, M. Barbero, C. Graby, T. Huynh. *Ortho Clinical Diagnostics, Rochester, NY*

Background:

The new VITROS XT Chemistry Products Slides* (UREA-CREA, ALTV-AST, TRIG-CHOL, ALB-TP, GLU-Ca, and TBIL-ALKP) have been developed with dual test capability for use on the VITROS XT 7600 Integrated System. The analytical performance of these new XT tests* has been evaluated for analytic quality using the sigma metrics methodology.

This series of new VITROS XT Chemistry Products Slides* have been developed with decreased test element size to enable dual test capability. The smaller test size supports reduced sample size and enhances operational efficiency by placing two assay test elements on a single slide. This is accomplished while maintaining analytical performance for accuracy and precision to support the assay quality expected from VITROS Products and Systems.

Methods:

For precision determinations, the total within-lab precision was calculated with quality control and patient samples using two replicates per day, twice per day over 20 days (total n=80) following CLSI EP5 guidelines.

For bias determinations, two different methods were used. For the first method, over one hundred patient samples spanning the measuring range were analyzed in singleton on a VITROS XT 7600 System against a reference method. The data was analyzed following CLSI EP9 guidelines using a Passing-Bablok regression, and the percent bias was calculated from the regression line. The second method used the College of American Pathologists (CAP) 2018 Chemistry survey fluids to calculate the percent bias.

Two different allowable total error (TE_a) values (Ricos and CLIA) were used to calculate the sigma metrics using the coefficient of variation (CV) and bias as determined above: $\sigma_{metrics} = (TE_a - \%Bias)/CV$.

Results:

Using the regression equation from the patient samples to determine Six Sigma quality, six of the XT tests* (UREA, TRIG, GLU, TBIL, ALTV and AST) had greater than six sigma performance versus the Ricos TE_a. Another three XT tests* (CREA, CHOL and ALKP) had greater than five sigma performance. The remaining three XT tests* (Ca, ALB and TP) had less than three sigma performance due to the very small Ricos TE_a (2.55%, 4.07%, and 3.63% respectively). When evaluating the assay quality versus the CLIA TE_a requirement, all XT tests* except ALB had greater than six sigma performance and ALB had greater than five sigma performance. The results were substantially equivalent when the sigma metric were determined using the CAP survey fluids for the bias estimate instead of the regression equation.

Conclusions:

The data presented here demonstrate that the new VITROS XT MicroSlides* on the VITROS XT Systems show excellent analytical performance for precision and accuracy when judged using the Sigma Metrics methodology against the CLIA TE_a requirement.

*Under development

B-334

Simple, Rapid and Sensitive Detection of Bacteria and Viruses with a Non-Amplification Nucleic Acid Detection Method

N. Kawada[1], K. Nakaishi[2], S. Watabe[2], M. Okamatsu[3], T. Yoshimura[4], E. Ito[1]. *[1]Waseda University, Tokyo, Japan, [2]TAUNS Laboratories, Inc., Shizuoka, Japan, [3]Hokkaido University, Hokkaido, Japan, [4]Health Sciences University of Hokkaido, Hokkaido, Japan*

Background: The detection of pathogenic bacteria and viruses have been conducted in specific laboratories using specimens obtained from patients or animals. There are two large issues. One issue is a transfer of specimens from the location of patients or animals to the laboratory, and the other one is critical drawbacks of PCR usually used as a detection method. The former may cause the spread of diseases, and thus an on-site detection method is required. The latter includes many problems of PCR, for example (1) non-specific or false positive amplifications, (2) volume limit for a target sample, (3) deactivation of enzymes used, (4) complicated techniques, (5) difficulty in designing probe sequences, (6) expense, and so forth. In the present study, we propose a new method for detection of bacteria and viruses, in which the performance is available on-site and the detection is not performed by PCR but by thio-NAD cycling.

Methods: Nucleic acids, such as DNA and RNA, in bacteria and viruses were detected. The target DNA and RNA examined were DNA of a *Mycobacterium tuberculosis* specific protein, MPB64, and RNA of an avian influenza A virus HA gene, respectively. For this purpose, we combined nucleic acid hybridization and thio-NAD cycling. The nucleic acid probes, which were linked with FITC, hybridized to the target sequences in the nucleic acids. The anti-FITC antibody linked with ALP was applied, and then a cycling reaction was conducted by a dehydrogenase (3α-hydroxysteroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androsterone phosphate). We then measured accumulated thio-NADH at the absorbance of 405 nm with a microplate reader. That is, our new method does not amplify the target nucleic acids but amplify the signals, and thus it is referred to as a "non-amplification nucleic acid detection method".

Results: We reached the limit of detection (LOD) of 10^5 copies/assay (i.e., 10^3 copies/µL) and the limit of quantification (LOQ) of 10^6 copies/assay (i.e., 10^4 copies/µL) for the single strand. Using the double strand, the LOD was 10^6 copies/assay (10^4 copies/µL), and the LOQ was 10^7 copies/assay (10^5 copies/µL). Using the plasmid, the LOD was 10^5 copies/assay (10^3 copies/µL), and the LOQ was 10^6 copies/assay (10^4 copies/µL). Further, we are now attempting to produce the exclusive detection machine, which can be used on-site.

Conclusion: Because the protocol of washout is included in our method and the measurement volume is larger than PCR, the possibility of false positive or negative results is decreased. The deactivation of enzymes can be avoided within the conditions described above. Further, because this method does not need DNA amplification,

designing probe sequences is not restricted by amplification products, and so it is easier and more flexible to design them. Thus, we can deal with high mutation rates of RNA viruses. In addition, we can detect samples by only using a small microplate reader, so that we do not need to use the expensive device for PCR. Therefore, our new method overcomes every difficulty of PCR.

B-335

Verification of Reference Intervals of Common Biochemical Analytes on Alinity ci in Karachi, Pakistan

F. Z. Kanani, M. Zubairi, U. Ata, A. H. Kazmi. *The Indus Hospital, Karachi, Pakistan*

Background :

Alinity ci was recently launched internationally by Abbott Diagnostics. We were amongst the first to install these analyzers on Accelerator a3600 track in Pakistan. It is imperative for the labs to verify reference ranges before adopting any assay. This is especially important in our setting as the reference intervals are generally established on the Western population, and local studies on Alinity are absent.

We aimed to verify the reference intervals of routine clinical chemistry and immunoassay analyzers on Alinity ci in the local population according to CLSI EP 28 A3.

Material and Methods:

After informed consent, healthy, voluntary blood donors were screened on the basis of a standard questionnaire and physical examination during regular blood drives. Additional 5 cc blood was taken for the verification of reference ranges. Each analyte was tested in 20 samples on two analyzers. If more than two of twenty values were outside the suggested reference range, the study was repeated on another twenty samples. The analytes included were urea, creatinine, sodium, potassium, chloride, bicarbonate, total and direct bilirubin, alanine transaminase, aspartate transaminase, alkaline phosphatase, gamma glutamyl transferase, uric acid, magnesium, phosphorous, calcium, amylase, CK, LDH, iron, total protein, albumin, C Reactive Protein, FreeT3, Free T4, TSH, prostate surface antigen, ferritin, Vitamin B12, Vitamin D, folate, AFP, beta hCG, FSH, LH and prolactin.

Results:

The mean (SD) age of the study participants was 37 (8.4) years. Mean weight was 70.2 (14.4) kg and haemoglobin concentration was 14.9(1.27) gm/dl.

Manufacturer suggested reference itervals were verified for 23 clinical chemistry and 11 immunoasssay parameters. Vitamin B 12 ranges fell below the criteria when determined on initial 40 samples. The study was repeated on another 20 samples. Out of a total of 60 healthy blood donors, 22 had vitamin B12 levels less than 187-883 ng/ml. This was understandable as vitamin B12 deficiency is very common in South East Asia, even amongst apparently healthy subjects as evidenced by literature. As expected, vitamin D levels (6.3 to 39.1ng/ml) were also at sub optimal levels in our population.

Conclusion: Manufacturer suggested reference intervals were verified on Alinity ci.

B-336

Evaluation of Newly Developed Concentrated Reagents for Clinical Chemistry Analyzers

T. Yamauchi, K. Shimamura, S. Yamashita, T. Yokoyama, H. Matsui. *Department of Clinical Laboratory, Kumamoto University Hospital, Kumamoto, Japan*

Background: The use of concentrated reagent is expected to reduce the frequency of reagent replacement, delivering the improved efficiency in laboratory operations with ever-growing demand. Concentrated reagents applicable for a clinical chemistry analyzer are being developed by JEOL Ltd. In this system, concentrated reagents are diluted with water in the reagent probe when the aspirated reagents are dispensed into the reaction cuvettes. We evaluated prototype concentrated reagents for calcium (Ca), uric acid (UA), creatinine (CRE) and total bilirubin (T-BIL) as a commissioned study in Kumamoto University Hospital.

Method: BioMajesty JCA-BM6070 (JEOL Ltd.) was used as a clinical chemistry analyzer. Following basic performances of reagents were evaluated; within-run precision, day-to-day precision, lower detection limit, linearity, interferences, and correlation with existing reagents. Anonymized patient samples obtained in the clinical laboratory of Kumamoto University were used. The use of the samples for this purpose was approved by the institutional ethics committee, and the study was performed in accordance with the Declaration of Helsinki.

Results: Coefficients of variation (CV) of within-run and day-to-day precision were less than 1.0%. Lower detection limits were 0.08 mg/dL for Ca, 0.04 mg/dL for UA, 0.011 mg/dL for CRE and 0.03 mg/dL for T-BIL, respectively. Linearities were given up to 20.0 mg/dL for Ca, 74.2 mg/dL for UA, 77.9 mg/dL for CRE and 104.0 mg/dL for T-BIL. Hemolysis slightly interfered UA, CRE and T-BIL. Turbidity interfered UA and T-BIL. Conjugated bilirubin interfered UA and CRE. Unconjugated bilirubin interfered UA and CRE. The correlation of coefficient between concentrated and existing reagents are 0.9831 for Ca (n=582), 0.9995 for UA (n=427), 0.9998 for CRE (n=693) and 0.9975 for T- BIL (n=729).

Conclusion: From the evaluation of the series of concentrated reagents, we confirmed that the developed reagents achieve comparable performance with existing reagents that are commonly used in Japan. At the same time, the use of concentrated reagent contributed to the increase of available test numbers per reagent container compared to exiting reagents. This will reduce the burden on the laboratory routine work.

B-337

How We Can Improve Productivity by Making Changes in Layout of a Clinical Analysis Laboratory

J. R. OLIVEIRA, K. S. Oliveira, I. B. W. Escalante, P. G. G. Costa, L. C. LOPES, G. R. Martins. *SABIN MEDICINA DIAGNOSTICA, BRASILIA, Brazil*

Background: The objective of the Production Management department in a clinical laboratory is to improve productivity by decreasing waste, reducing turnaround time of production and automatizing processes. In order to do that we apply the principles of Lean Manufacturing. Lean is the systematic method of reducing waste, whereas waste is anything that is in excess during production and does not add value to the final product. Our production department developed several projects in the year of 2018 related to changes in layout. In total there were 12 projects, from those, nine were executed in Brasilia, and three were developed around our 14 regional branches. In all of these projects we applied lean manufacturing delivering a final product that consists in a more efficient line of production, reducing the amount of steps needed to perform daily activities and routine processes.

Methods: In order to exemplify this type of project we are going to talk about two successful experiences we had last year. The first example shows the application of lean principles in a Cytogenetics Department. This department was recently created and was located in an area that was not designed for its needs. We visited the site and studied their process in order to understand the workflow, later, we suggested a new layout. At first, it took the staff a total of 25 steps to conclude their routine and many of those steps overlapped each other creating a scrambled path map. After we studied and improved their process, it only took 16 steps to conclude the same tasks as before, and the workflow was much more fluid and organized. The second example was executed in the emergency laboratory. In order to improve the flow inside the technical area, the same principles were applied. Before our intervention, the four different types of samples crossed paths multiple times and the start and finish points of the process were not ideal. Upon analyzing the time-motion study, we arrived on a new and improved layout. On this layout, we reorganized the equipment in a sequential order, in a way that different samples could have a clear path throughout the area without overlap. This allowed for a better flow for the technical staff and it took less steps to conclude the processes.

Results: As a result, we gained productivity through the reduction of time needed to perform tasks; we also reduced risk of accidents, and the total distance each employee had to walk on a daily basis.

Conclusion: In conclusion, by analyzing the production process, we could optimize the clinical laboratory and deliver results in less time. By reducing the total distance the employees had to walk during the day, we provided a better rested staff, less prone to work-related injuries and less likely to take sick days. We also provided a better and more ergonomic environment for the staff. Our goal with these types of projects is the continuous improvement in both quality and agility in order to deliver better and faster results for our clients.

B-338

How to be Fast and Efficient with the Changes in Clinical Analysis Laboratory: "The Beginning and Development of a Production Department."

J. R. OLIVEIRA, I. B. W. Escalante, P. G. G. Costa, K. S. Oliveira, M. M. A. Lima, G. R. Martins. *SABIN MEDICINA DIAGNOSTICA, BRASILIA, Brazil*

Background: It takes a lot of humility to admit that your process is not good enough or efficient enough. In the competitive world of today's clinical analysis laboratories, we have to accept the fact that constant change is not only necessary, but also vital to the endurance of the business. In our company, the development of projects has recently been reevaluated and the process of implementing new tests and techniques was changed and improved significantly. Our Production Department or Project Management Office (PMO) is still at a very young age and we are still learning with our mistakes and always studying new ways on how to manage our projects. The function of the PMO is to ensure that the raw materials turn into a final product in an effective and smooth manner. This department has to constantly work to minimize costs, expenditures and excesses in waste. For that, we frequently use tools of Lean philosophy and Kaizen in order to better the production while decreasing unnecessary waste of resources, people and time. Our main objective is to minimize costs and deliver a better final product. All our efforts go towards improving productivity, be it by acquiring better equipment, rethinking processes, altering workflows and training teams to operate in a more efficient way.

Methods: With a team of two people, we were able to manage 138 projects during the years of 2017 and 2018 utilizing the principles of Lean Manufacturing. We developed our projects according the PMBOK Guide (Project Management Body of Knowledge). According to the PMBOK Guide, Project Management is the application of knowledge, abilities, tools and techniques in the activities related to the project in order to attend to its requisites. The project management activities are divided in five groups of processes: initialization, planning, execution, monitoring- controlling, and finalization.

Results: Since its creation, the Production Department has managed 138 projects, those, being 118 developed in Brasilia, on the main branch of the laboratory, and the other 20 developed across the country, in some of our 12 regional branches. From the 118 projects that were developed in the main branch, 96 of them were related to new equipment or new tests, and the remaining 22 were related to changes in layout and/or continuous improvement.

Conclusion: By using the techniques of PMO and improvement tools we have developed and monitored several successful projects that have overall improved the operations in a clinical laboratory proving that a Production Department or Project Management Office is of great importance in any business. So far, this department has only worked with the technical areas of the laboratory, but our mission is to expand our reach and apply our recently acquired knowledge and expertise to every department of the company, propitiating a dynamic environment where everybody´s goal is to better the processes and operations of the entire corporation continually.

B-339

Automatic Repetitions by Delta Analysis of the Sodium Test from June to August 2018

B. F. Camara[1], P. S. Elias[1], C. T. Trujillo[1], F. E. Ciamponi[2], P. S. Osorio[1]. [1]*Diagnostico da America (DASA), Barueri, Brazil,* [2]*State University of Campinas (UNICAMP), Campinas, Brazil*

Background: Each day, the number of exams performed in large-scale diagnostic medicine laboratories increases considerably. Such volume of information is impractical for each exam to be manually curated by human professionals and approved in a timely manner to be of practical use in health facilities.The automated system of production provides automated results analysis tools, among them, a delta analysis, which allows to establish the different sources through the analysis of the obtained data, comparing with the current result, once previously established. In this way, the decision rules of the system for the repetition of a command by delta analysis, such as an automatic repetition, become viable. **Methods:** Data were extracted from the automated system of production, considering the Sodium test performed in a laboratory of clinical analyzes between the period from June to August 2018. The percentage of automatically repeated results was evaluated based on the criterion of the delta analysis, considering the variation of 4.0% compared to the previous results released in the 7-day period. **Results:** During this period, 58,256 samples were analyzed and 4194 were repeated. Among the repetitions we had 409 (9.75%) samples repeated automatically due to delta analysis (Table 1).

Conclusion: The data obtained suggest a gain in agility in the repetition request process, since on average 10% of the repetitions in this period would be awaiting manual evaluation of a biochemist. In addition, the automatic decision adds security to the process, since all repetitions were directed towards a higher level of release, performed by a medical doctor because of delta analysis.

Table 1: Automatic repetitions by delta analysis

	Jun/18	Jul/18	Aug/18	Total
Performed tests	18531	18840	20885	58256
Repetitions	1236	1770	1188	4194
Repetitions by delta analysis	82	172	155	409

B-340

The New Liquid Fructosamine Assay for the ARCHITECT cSystems Instrument

T. M. Alligrant[1], J. De Giorgio[2], F. Andreoletti[2], S. Williams[1], R. Lucini[2], A. Cugini[2], F. Vespasiani[2], M. Valdambrini[2], J. Salazar[1], K. Bachhawat[1], M. Beischer[1]. [1]*Abbott Laboratories, Irving, TX,* [2]*Sentinel Diagnostics, Milan, Italy*

OBJECTIVE: To present the performance and interference test results of the new Fructosamine assay on the ARCHITECT *c*Systems instruments.

RELEVANCE: The new Fructosamine assay (list number [LN] 03R05) is now liquid, ready-to-use, with a longer calibration interval. Additionally, during development, more potential interferents were evaluated relative to the previous MULTIGENT Fructosamine LN 06K94 assay.

METHODOLOGY: The Fructosamine assay (LN 03R05) utilizes the same NBT/formazan colorimetric methodology as the previous Fructosamine 06K94 assay. Briefly, fructosamine, in its ketoaminic form, reduces nitroblue tetrazolium (NBT) to formazan in an alkaline medium. The reaction rate, photometrically measured at 548 nm, is directly proportional to the concentration of fructosamine in the sample.

VALIDATION: The table below displays the performance characteristics of the new Fructosamine assay (LN 03R05) relative to the previous assay LN 06K94. The endogenous and Intralipid interference results for the new Fructosamine 3R05 assay only is displayed in the lower portion of the table. All interference data shown, represent the highest acceptable interference levels.

<table>
<tr><th>Characteristic</th><th colspan="4">LN 06K94</th><th colspan="4">LN 03R05</th></tr>
<tr><td>Configuration</td><td colspan="4">Lyophilized, one-reagent assay</td><td colspan="4">Liquid, Ready-to-Use, two-reagent assay</td></tr>
<tr><td>Imprecision</td><td>N</td><td>Mean (µmol/L)</td><td>Total SD (µmol/L)</td><td>Total %CV</td><td>N</td><td>Mean (µmol/L)</td><td>Within-Lab SD (µmol/L)</td><td>Within-Lab %CV</td></tr>
<tr><td></td><td>40</td><td>190.5</td><td>8.42</td><td>4.42</td><td>88</td><td>22</td><td>1.6</td><td>7.1</td></tr>
<tr><td></td><td>40</td><td>314.0</td><td>9.22</td><td>2.94</td><td>88</td><td>285</td><td>7.8</td><td>2.7</td></tr>
<tr><td></td><td>40</td><td>547.9</td><td>11.91</td><td>2.16</td><td>88</td><td>497</td><td>9.1</td><td>1.8</td></tr>
<tr><td></td><td>-</td><td>-</td><td>-</td><td>-</td><td>88</td><td>917</td><td>8.6</td><td>0.9</td></tr>
<tr><td>Method Comparison:</td><td colspan="4">06K94 vs. Comparative Method</td><td colspan="4">03R05 vs. 06K94</td></tr>
<tr><td></td><td>N</td><td colspan="3">129</td><td>N</td><td colspan="3">130</td></tr>
<tr><td></td><td>R</td><td colspan="3">0.979</td><td>R</td><td colspan="3">0.747</td></tr>
<tr><td></td><td>Equation</td><td colspan="3">Y = 1.03x + 9.20</td><td>Equation</td><td colspan="3">[03R05] = 1.09[06K94] + 26.30</td></tr>
<tr><td></td><td>Range (µmol/L)</td><td colspan="3">112 – 784</td><td>Range (µmol/L)</td><td colspan="3">15 – 672</td></tr>
<tr><td>Calibration Stability in hours</td><td colspan="4">72 (3 days)</td><td colspan="4">168 (7 days)</td></tr>
<tr><td>Interferent</td><td colspan="2">[Interferent]</td><td colspan="2">[Fructosamine] (µmol/L)</td><td colspan="2">Difference (µmol/L)</td><td colspan="2">% Diff.</td></tr>
<tr><td>Bilirubin (Conjugated)</td><td colspan="2">23.0 µmol/L</td><td colspan="2">181</td><td colspan="2">16</td><td colspan="2">8.86</td></tr>
<tr><td></td><td colspan="2">79.3 µmol/L</td><td colspan="2">550</td><td colspan="2">53</td><td colspan="2">9.61</td></tr>
<tr><td>Bilirubin (Unconjugated)</td><td colspan="2">32.1 µmol/L</td><td colspan="2">195</td><td colspan="2">16</td><td colspan="2">8.21</td></tr>
<tr><td></td><td colspan="2">72.2 µmol/L</td><td colspan="2">573</td><td colspan="2">41</td><td colspan="2">7.22</td></tr>
<tr><td>Glucose</td><td colspan="2">46.64 mmol/L</td><td colspan="2">169</td><td colspan="2">17</td><td colspan="2">10.05</td></tr>
<tr><td></td><td colspan="2">133.40 mmol/L</td><td colspan="2">524</td><td colspan="2">46</td><td colspan="2">8.83</td></tr>
<tr><td>Hemoglobin</td><td colspan="2">1.44 g/L</td><td colspan="2">168</td><td colspan="2">5</td><td colspan="2">3.09</td></tr>
<tr><td></td><td colspan="2">11.90 g/L</td><td colspan="2">493</td><td colspan="2">38</td><td colspan="2">7.78</td></tr>
<tr><td>Human Triglycerides</td><td colspan="2">5.87 mmol/L</td><td colspan="2">141</td><td colspan="2">13</td><td colspan="2">9.19</td></tr>
<tr><td></td><td colspan="2">10.99 mmol/L</td><td colspan="2">410</td><td colspan="2">34</td><td colspan="2">8.38</td></tr>
<tr><td>Intralipid</td><td colspan="2">6.25 g/L</td><td colspan="2">145</td><td colspan="2">-10</td><td colspan="2">-7.16</td></tr>
<tr><td></td><td colspan="2">18.75 g/L</td><td colspan="2">429</td><td colspan="2">-32</td><td colspan="2">-7.51</td></tr>
<tr><td>Uric Acid</td><td colspan="2">1.51 mmol/L</td><td colspan="2">184</td><td colspan="2">3</td><td colspan="2">1.55</td></tr>
<tr><td></td><td colspan="2">1.55 mmol/L</td><td colspan="2">507</td><td colspan="2">16</td><td colspan="2">3.24</td></tr>
</table>

CONCLUSIONS: The new Fructosamine LN 03R05 assay displays enhanced performance characteristics and improved ease of use over the predicate Fructosamine LN 06K94 assay.

B-341

Evaluation of Precision of the New Multi-Test VITROS® XT Chemistry Products Slides* with the Application of Novel Algorithms on the VITROS® XT 7600 Integrated System

J. Miller, T. DiMagno. *Ortho Clinical Diagnostics, Rochester, NY*

Background:

Ortho Clinical Diagnostics (Ortho) has developed the new VITROS® XT 7600 Integrated System (VITROS XT 7600 System) which utilizes digital chemistry technology to support the new VITROS XT Chemistry Products Slides* (XT MicroSlides). The XT MicroSlides with dual test capability are intended to reduce sample size and enhance operational efficiency while maintaining analytical performance versus the conventional single slide test. Due to small sample volume, the size of the MicroSlide region available for optimum analytical measurement is reduced. To mitigate against possible performance sensitivities, the VITROS XT 7600 System utilizes Digital chemistry technology. Digital chemistry uses wavelength-specific LED technology to capture images of MicroSlides on the Digital Imaging Reflectometer. Digital chemistry technology enables the use of imaging algorithms to improve chemistry results, such as within-lab precision, by ensuring that the optimal region of the sample is read even in the presence of variation in metering position. This study will examine within-lab precision, comparing the VITROS XT 7600 System with digital chemistry capability versus the VITROS 5600 Integrated System (VITROS 5600 System) with traditional spectrophotometry.

Methods: The precision of six XT MicroSlides*: UREA-CREA Slides, ALTV-AST Slides, TRIG-CHOL Slides, ALB-TP Slides, GLU-Ca Slides, and TBIL-ALKP Slides for two concentrations was evaluated using quality control materials on a VITROS XT 7600 System. Total within-lab precision (reported as percent coefficient of variance) for a single calibration was evaluated with two runs per day with two replicates per run over 20 days, for a total of 80 replicates following CLSI EP05 guidelines. The within-lab precision for XT MicroSlides* on the VITROS XT 7600 System was compared to the corresponding single test slides within lab precision on the VITROS 5600 System.

Results: The XT MicroSlides* lots reported within-lab precision as improved or equal for 19 out of 24 examined assay/fluid combinations. The exceptions being CHOL at PV1 (1.9% vs. 1.5%); CREA at PV2 (1.6% vs. 1.4%); TBIL at PV1 (3.8% vs. 3.1%); ALTV at PV1 (2.4% vs. 1.9%); and ALTV at PV2 (1.7% vs. 1.4%).

In addition, customer quality control precision data from single test slides from VITROS 5600 Systems were extracted and evaluated. The customer quality control data points were screened for statistical outliers and outliers were removed. The %CV was calculated for each PV fluid/reagent lot/system combination and compared to the within-lab precision for the XT MicroSlides* on the VITROS XT 7600 System.

Conclusion: The data presented here demonstrate that the new XT MicroSlides* using new imaging algorithms provide comparable or improved precision relative to single test slides from internal and external precision studies.

B-342

Precise Sample Pipettor

T. Mizutani[1], H. Narita[2], K. Umebara[2]. [1]*Beckman Coulter Inc, Chaska, MN,* [2]*Beckman Coulter Inc, Mishima, Japan*

Introduction: Precise and accurate delivery of patient sample is a critical step in obtaining accurate test results. Automated analyzers have improved precision over the years, but there is still room for improvement. Additionally, there is a desire to conserve sample collected from patients, leading to a need for even smaller volume sample delivery. This team set out to develop motion profiles that would achieve very high precision (≤1% CV) for small volume delivery and maintain fast throughput while eliminating sample carryover by employing disposable pipette tips.

Method: A colorimetric method for determining precision was employed, using varying volumes of a known concentration of colored dye as a sample and pipetting it into a known volume of 7% bovine serum albumin solution. Concentration of the dye was calculated, and measurements of the delivery were performed using a spectrophotometer. Three different motion profiles were created for varying ranges of volume delivery (250 µL, 25-100 µL and 2-24 µL targets). All tests, with 6 different target values at both high and low viscosity, were conducted with 10 replicates per sample, using an 8 second pipetting cycle, on 5 different pipetting systems.

Results: Coefficients of variation (CV) of less than 1% for all samples were achieved by incorporating these new pipetting profiles.

Precision profile summaries by target value

Conclusion: The three pipetting motion profiles are meeting and, in some cases, exceeding expectations for precision using the new pipetting system and disposable tips. This performance, particularly at volumes <25 µL, will allow for use of smaller patient sample volumes without sacrificing assay performance.

B-343

Correlation of Common Clinical Analytes between Cobas c311 and Alinity c in Karachi, Pakistan

F. Z. Kanani, A. H. Kazmi, M. Zubairi. *The Indus Hospital, Karachi, Pakistan*

Background: Alinity series has been recently launched internationally by Abbott Diagnostics. We were amongst the first in Pakistan to install Alinity ci on a3600 track as part of Total Laboratory Automation. As part of the evaluation studies, we compared results of twenty four common clinical chemistry analytes between cobas c311 and two Alinity c analyzers. To our knowledge, this is the first method comparison study across a range of analytes between cobas and Alinity clinical chemistry platforms in Pakistan.

Material and Method: Two Alinity c analyzers were evaluated separately for twenty four clinical chemistry analytes. Forty samples of every analyte were run on cobas c311, and within two hours of testing were analyzed on an Alinity c. The method comparsion study lasted a minimum of eight days, and values spanned the entire analytical measuring range. Correlation between cobas c311 and Alinity c was calculated by Passing Bablok, Deming regression and regular regression analysis. Slope, intercept and correlation coefficient were calculated. EP evaluator was used for data analysis.

Results: The method comparision study showed good correlation between the cobas c311 and Alinity c for all the analytes tested. Correlation coefficient (r) was greater than 0.99 for all parameters except HDL cholesterol which showed an r= 0.98 for both Alinity c, and albumin which showed r= 0.98 for one Alinity c. (Table attached). Passing Bablock and Demings regression curves obtained showed results on both the primary and secondary analyzers to be statistically similar.

Conclusion: There is very good correlation for clinical chemistry analytes between cobas c311 and Alinity c.

		Alinity1			Alinity 2		
X method	Analyte	Slope	Intercept	Corr Coef (r)	Slope	Intercept	Corr Coef (r)
Cobasc311	Albumin	0.96	0.0296	0.9667	1.015	-0.10078	0.9969
Cobasc311	ALP	0.993	-1.47799	0.9965	1	-O.906	0.9994
Cobasc311	ALT	1.016	0.64929	0.9999	1.025	-0.21677	0.9996
Cobasc311	Amylase	1.005	-0.12411	0.9996	1.005	-0.23342	0.9999
Cobasc311	BUN	0.996	-0.0217	0.9991	0.995	0.31	0.9995
Cobasc311	Calcium	1.002	-0.06154	0.9963	1.002	-0.05114	0.9965
Cobasc311	Cholesterol	1.023	-1.688	0.9975	1.006	3.367	0.9959
Cobasc311	Creatinine	1.003	-0.02133	0.9996	1.008	0.05799	9999
Cobasc311	Bilirubin (d)	0.965	0.11525	0.9955	0.983	0.13313	0.9961
Cobasc311	GGT	0.995	2.1	0.9996	0.996	2.031	0.9998
Cobasc311	Glucose	1	0.145	0.9992	1.008	-0.529	9990
Cobasc311	HDL c	1.024	-0.428	0.9634	1.045	-O.81745	0.987
Cobasc311	Uric acid	0.99	0.04801	0.9995	0.994	0.05311	9994

B-344

A Novel Ultra-Sensitive Multiplex Immunoassay Platform

D. Moreno[1], T. Saito[2], S. Kamer[1], A. Juang[1], H. Yu[1], K. Choudhury[1], L. Carbonell[1]. [1]*MagArray Inc, Milpitas, CA,* [2]*Hitachi High-Technologies, Hitachinaka-shi, Japan*

Background: MagArray platform is based on the detection of magnetic particles as labels in bioassays. The detection of magnetic and electric signals provides unique advantages over traditional immunoassay platforms based on optical signals. For example, the signals are insensitive to matrix and common interferences, specifically reported here is the high sensitivity of multiplex immunoassays allowed by this platform. **Objective:** Detection of IL6, IL8, IFN γ and MIP-1α with a sensitivity below 0.1 pg/ml is used to demonstrate the potential of magnetic biosensors for multiplex immunoassays with high sensitivity. **Methods:** Antibody pairs for the four cytokines were first screened in a parallel mode to quickly identify antibodies with high affinity and minimal cross reactivity. After assay optimization, the sensitivity and dynamic ranges are evaluated according to the CLSI guideline. **Results:** In this multiplex assay of four cytokines, the background or blank signals are caused mainly by the multiplex format using multiple antibodies. The limits of blank were 0.04 pg/ml, 0.05 pg/ml, 0.05 pg/ml, and 0.05 pg/ml for IL6, Il8, IMP-1α, and IFN-γ, respectively. At 0.1pg/ml of each protein in the multiplex assay, the CVs were 7.9%, 6.9%, 10.7%, and 14.5% respectively. We are also applying the multiplex immunoassay described here to test a small scale of lung cancer (NSCLC) samples to evaluate their potential for early diagnosis of lung cancer combining with other biomarkers we have developed. **Conclusions:** MagArray platform is demonstrated to be an appropriate platform for immunoassays that require both high sensitivity and multiplexity. A sensitivity of <0.1pg/ml is routinely demonstrated in a multiplex configuration. In addition, the detection of magnetic signals, rather than optical signals, offers a unique benefit for protein detection in complex biological matrices.

B-345

Establishing and Validating Product Performance Characteristics

V. H. Petrides, R. Scopp, A. Orzechowski. *Abbott Laboratories, Abbott Park, IL*

Background: *In vitro* diagnostic (IVD) manufacturers are responsible for designing, producing, and delivering high quality, safe, and effective products. However, the design and development processes used to optimize and deliver robust performance of devices can vary greatly among manufacturers. Unfortunately, manufacturers do not always share this information, leaving laboratorians to trust that manufacturers have comprehensively tested and evaluated their products prior to releasing them into the market. This poster provides an overview and an example of one approach manufacturers may take to ensure the quality, safety and effectiveness of their devices.

Methods: When designing and developing IVD products, manufacturers should consider the entire product life cycle - from inception to retirement. Initially, user needs such as the patient intended use population, the product use-setting / intended users and the clinically / medically relevant analytical goals are identified. Product design and performance requirements such as product size, turn-around-times, and allowable error specifications are then determined based on these user needs. From these elements, design controls are established to optimize performance and maintenance over time. After design controls are established, the product design and performance requirements are verified and validated. Once the product is placed on the market, the manufacturer monitors the manufacturing process, stability, and product performance internally and in the field. Throughout the life cycle, risk assessments are performed to identify potential weaknesses in the process, test methods, and use of the product. If issues are identified, improvements to the process, process controls, or production testing may be made. Eventually, as technology changes and standards of care evolve, a product will be retired. **Results:** The product life cycle process for a hypothetical HbA1c assay is illustrated. Examples of user needs, analytical performance goals and how these goals could be translated into product design and performance requirements are provided. Studies to verify and validate requirements are discussed. Error budgeting, risk assessment, and monitoring are also addressed.

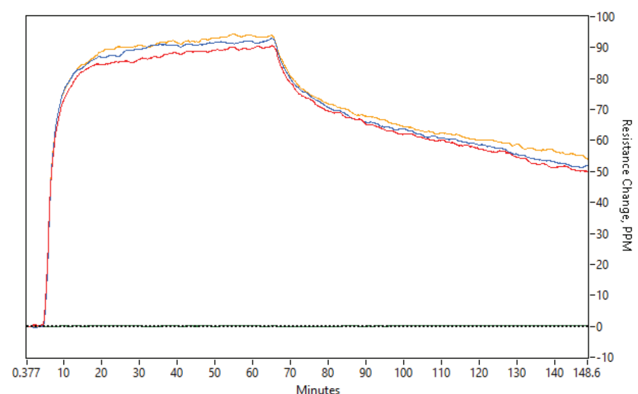
Conclusion: Manufacturers should be willing to share the process they follow to design, produce, and deliver IVD products, as doing so can better assure laboratorians that the products in their lab have been developed using rigorous methods demonstrating high quality, safety, and effectiveness and provide valuable information for the maintenance of their own laboratory.

B-346

Magnetic Biosensors as a Novel Platform for Characterizing Protein-Protein Interactions

H. Yu[1], S. Osterfeld[1], K. Choudhury[1], H. Mizukami[1], L. Carbonell[1], S. Wang[2]. [1]*MagArray Inc, Milpitas, CA,* [2]*Stanford University, Palo Alto, CA*

Background: MagArray platform is based on the detection of magnetic particles as labels in bioassays. Since the biosensors are designed to detect magnetic particles only when particles are bound or captured to the sensor surface, this proximity detection mechanism allows the real-time monitoring of the binding signals. We report here that MagArray platform can be used to probe protein-protein interactions and provide kinetics parameters including association and rate constants. **Objective:** A feasibility study of the application of magnetic biosensors in the field of protein-protein interactions. **Methods:** We used commercial TSh antibodies and proteins as the models to demonstrate the feasibility of measuring the kinetic parameters between TSh protein and antibodies using the magnetic biosensors. More specifically, TSh protein was fixed on the sensor surface by either physisorption or chemical conjugation methods. TSh antibody was then conjugated to magnetic beads through either chemical conjugation or secondary antibody bridging. We have developed a simple model for the curve fitting process of the real-binding curves obtained and calculated the dissociation and rate constants. In addition, we also demonstrated the measurement of kinetic parameters of bindings in complex matrix such as buffers spiked with different amount of serum. **Results:** A representative graph of binding curves is shown here. The calculated kinetic parameters are compared with the reported values. Uniquely, the magnetic biosensors provide reliable binding measurement in complicated matrices such as serum, and the matrix effect, as expected, is dependent on the amount of serum added. **Conclusions:** MagArray platform is a novel platform for monitoring protein or molecular binding in real time and determining the binding parameters. The capability of monitoring the binding in complex matrices also promises wide applications of this technology in the field of protein interactions that is unfeasible in the past.



B-347

Extending the Linear Reading Range of a Luminometer

D. Noll, B. Kozicki, R. Wolf, T. Mizutani. *Beckman Coulter, Chaska, MN*

Introduction: Chemiluminescent assays currently apply complicated math models to define calibration curves and fit varies by lot with these models. Photo-detectors employ a conventional approach of photon counting, which is hampered at high count rates due to non-linearity at the upper limit. This non-linearity is a result of the limits of pulse-pair resolution, defined as the minimum time interval at which each individual pulse can be discriminated from another. As light concentrations increase, multiple pulses begin to be counted as a single pulse. To avoid this counting saturation, an alternate approach is to use analog measurements for determining light intensity, which is a better method at high light concentration, but not at low levels. An approach to combine the traditional photon counting method with the analog measurement method was developed for an automated immunoassay analyzer.

Method: An experiment was designed to test an existing chemiluminescence enzyme immunoassay, Access TSH 3rd IS using the photon counting methodology and compare performance to the new combined method. Calibrators were analyzed using a new prototype instrument; both the photon counting method and the new combined method were tested. Both RLU signal and linearity of the calibration curves were compared. The new combined method used 12 million Relative Light Units (RLU) as the crossover point: the photon counting method used below this point and the analog measurement method used above this point.

Results:

The table below depicts RLUs generated at each of the calibrator levels, for both methods. Note the lower RLU for the zero-analyte calibrator and higher RLUs for the analyte containing calibrators when using the new combined method and hardware.

Calibrator	Concentration (µIU/mL)	RLU with photon counting detector	RLU with New Detector
S0	0.00	8,651	6,646
S1	0.05	92,183	250,161
S2	0.30	507,796	1,417,782
S3	3.00	4,886,071	13,394,057
S4	15.0	21,671,666	69,324,524
S5	50.0	51,120,582	239,489,086

Conclusion: The new combined method and hardware successfully extended the useable range of RLUs by ~10 fold, from up to 40 million RLU to at least 400 million RLU as the upper limit before signal saturation. Linearity is maintained within the entire range of RLUs. The extended linear measuring range of the new combined method allows simpler curve fitting math models to be applied for calibration curves. More linear curves lead to more robust assay development.

B-348

Performance Characteristics of a Newly Developed Automated High-Throughput Biotin Method (RUO) on the ARCHITECT i2000SR

M. Matias, J. Prostko, K. Richard, J. Munoz, R. Schneider. *Abbott Diagnostics, Abbott Park, IL*

Background and Relevance: The use of biotin as a supplement has increased significantly in recent years and many health care professionals may not recognize their patients are taking high doses. The apparent increased use has also resulted in an increase in both reported adverse events to the FDA and case reports from health care professionals describing inaccurate lab results for certain assays that utilize a biotin-streptavidin free capture methodology. Despite these reports, overall awareness and education by laboratory professionals, the prevalence of biotin interference in patients presenting to hospitals or having routine blood work performed is still largely unknown. One limitation is the availability of a high throughput biotin assay that is capable of measuring supratherapeutic levels of biotin in blood. The purpose of this study was to characterize an automated research use only (RUO) method for biotin quantification on the ARCHITECT *i*2000SR. The importance of biotin prevalence during lab testing, particularly in acute care settings where critical lab results cannot be delayed will help medical professionals and health care systems estimate risk to their patient populations.

Materials and Methods: The ARCHITECT STAT Biotin assay is a competitive 2-step assay. In the first step, patient sample is added to paramagnetic particles coated with streptavidin. Biotin in the patient sample is bound by streptavidin coated paramagnetic particles in this first step, followed by a wash step to remove any non-specifically bound patient sample. In the second step, an acridinium labeled biotin conjugate is added, followed by another wash step and detection. Analytical studies (Detection Limits, Precision, Linearity, and Method Comparison) were carried out using prepared panels (created gravimetrically by adding USP grade biotin to normal human serum or plasma). Detection limits were determined by testing a 12-level panel in replicates of 10 on three separate days across two ARCHITECT i2000SR instruments for a total of 60 replicates per level. Precision was estimated over 3 days (10 reps per day) utilizing a Low, Medium and High control (Low = 9.8; Medium =15.7; High = 21.5ng/mL). Linearity and method comparison were assessed using prepared panels with biotin levels ranging from 0.0 – 50.0ng/mL and LC-MS/MS.

Results: The limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantification (LoQ) were 2.8, 4.4, and 5.0ng/mL; respectively. Precision estimates for the Low, Medium and High controls were 11%, 6.3%, and 5.2%; respectively. Percent deviation from linearity was within 20%, between the ranges of 5.4 - 48.8ng/mL. Accuracy by LC-MS/MS demonstrated an average percent difference of -0.5% (serum) and -21% (LiHep), between 5.0– 50ng/mL.

Conclusions: The ARCHITECT *STAT* Biotin assay is a fully automated high throughput method capable of delivering the first result within 15 minutes and expected maximum throughput of up to 150 tests per hour. Analytical performance of the Biotin assay was found to be acceptable for screening patient specimens for biotin interference at concentrations that have been reported to interfere with immunoassays that utilize biotin-streptavidin free capture methodologies.

B-349

Non-Invasive MRI for Assessment of Medical Grafts and Biomaterials In Vivo

D. A. Szulc, H. M. Cheng. *University of Toronto, Toronto, ON, Canada*

Biomaterial grafts are essential ingredients in many tissue engineering strategies, as they allow cells to penetrate, attach, and migrate; they retain biochemical factors conducive to tissue growth; and they biodegrade over time at a rate ideally matched to that of new tissue production. One difficult aspect to advancing this technology in the laboratory is determining the properties and fate of these materials once introduced in vivo. Non-invasive imaging technology such as MRI holds significant potential for monitoring implanted biomaterials. In this study, we investigate a novel approach to labelling biomaterials with a highly efficient and versatile T1 MRI agent, which allows for accurate and sensitive detection of a variety of different biomaterials in vivo. The MRI agent, MnP, was synthesized and characterized by H^1NMR, UV-spectroscopy, mass spectrometry, and high-performance liquid chromatography. The agent was then conjugated to a variety of materials including biological polymers such as collagen and synthetic such as polyurethane. Binding efficiencies, stability, and retention were assessed by UV and Flame Atomic Absorption Spectroscopy. All materials exhibited high retention rates, up to the point of base polymer degradation, while maintaining significant MRI signal on a 3.0T clinical scanner (Achieva 3.0 T TX, Philips Medical Systems). Animal studies were performed on five female adult Sprague Dawley rats (Charles River Laboratories) weighing 250-300g. Functionalized collagen hydrogels were injected subcutaneously, and animals were imaged on a 3.0T scanner. The injected materials were tracked longitudinally up to 22 days (Figure 1) and demonstrated significant signal until complete degradation. This promising new method for sensitive and accurate MRI tracking of biomaterials will aid assessment and development of novel materials for tissue engineering and regenerative medicine.

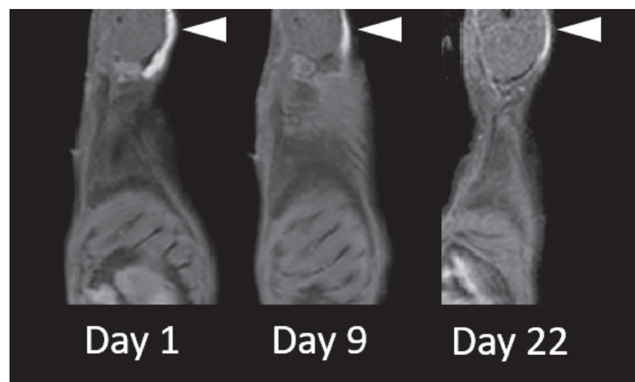


Fig 1. Sagittal fat-saturated T1-weighted spin echo images of a rat injected with a functionalized collagen gel. The gel observed, degraded over time, w

hich was evident by MR visualization and T1 signal.

B-350

Unique Application of Machine Vision in Future Automated Immunoassay Systems

A. Sawhney, M. Willette, N. Sovde, F. Arita, T. Mizutani. *Beckman Coulter, Chaska, MN*

Introduction: Conventional automated clinical diagnostic testing systems have various process monitoring functions (e.g. optical sensors, pressure sensors, thermistors, etc). These monitors check the integrity of instrument function, but most of those tools are limited to indirect sensing and do not directly monitor the critical elements of correct assay processing. This study examines the use of a new tool, machine vision, to directly monitor critical assay processing steps.

Method: Incorporating the new process monitoring includes insertion of imaging technologies with software algorithms. Combinations of placement, backlighting and multiple algorithms were studied to demonstrate capability of direct measurements of sample within pipette tips and reaction steps in the vessels. Four applications were pursued as follows:

1. Sample volume monitoring: image and software algorithms measure the distance from bottom of tip to sample meniscus, using pixels, then convert measurement to volume

2. Total reaction volume monitoring: image and software algorithms measure the distance from bottom of vessel to reaction meniscus, using pixels, then convert measurement to volume

3. Residual volume monitoring: image and software algorithms execute pattern matching and convert to residual volume

4. Particle retention monitoring: image and software algorithms execute measurement of gray-scale gradient and convert to particle concentration

Results: Summary of accuracy and capability of each of the 4 applications are listed below:

1. Sample volume detection range was demonstrated to be 2 to 100 µL with ± 5% accuracy capability

2. Reaction volume detection range was demonstrated to be 50 to 250 µL with ± 5% accuracy capability

3. Residual volume detection was demonstrated with a minimum volume of 15 µL capability

4. Particle retention range of 40-100% retention was demonstrated with ± 5% accuracy capability

Conclusion: This study confirms the performance of machine vision for direct measurement of various sample reaction volumes. Proactive and direct assessment will potentially permit future immunoassay systems to notify users of processing errors, permitting earlier detection and resolution, and lowering risk that erroneous but believable results will be reported.

B-351

The Real Time $O_2$and pH Measurement System for Cell Growing Monitoring on 24-Well Microplate

S. PARK. *Dongguk University, Goyang-si, Korea, Republic of*

Introduction: The aim of this study is to develop a real time measuring system to detecting the dissolved O_2 and pH in 24-well microplate. The Measuring of dissolved oxygen and pH change are very important parameters to understand a metabolism of cell on developing new drug. We used the commercial patch which is change an exciting time depend on concentration of oxygen, and pH. **Method:** In this study, we construct a detecting system which is mainly consist of LED and photodiode as seen Fig. 1. The LED play an activation of patch, and the photodiode paly a detecting a light source from patch. We describe an optical noise reduction algorithm to decrease the scattering light from microplate. To validate this algorithm, we used the commercial reagent for pH (6, 7, 8, and 9), and the dissolved oxygen was made with high purity oxygen (99.9% O_2) and distilled water. **Results:** A detected dissolved O_2 was 25±1.03%, 30±0.84% and 35±1.01%(mean±SD) for 25.02%, 30.03%, and 35.10% on controlled material respectively. A detected pH was 6±0.20, 7±0.15, 8±0.06, and 9±0.38 for 6, 7, 8 and 9 pH on controlled materials respectively. **Conclusion:** The algorithm will be used to usefulness of cell activation research such as developing new drug with mitochondria rules. In further study, we will test the real time O_2 and pH measurement system with live cell and improve the system to use a laboratory condition including user interface.

Figure 1. The real time O_2 and pH measurement system with 24-well microplate

B-352

Evaluation of the new Rochecobas e801 Immunoassay Analyzer

S. F. Yew, C. S. Lau, S. P. Hoo, T. C. Aw. *CHANGI GENERAL HOSPITAL, Singapore, Singapore*

Background: Roche diagnostics introduced a new immunoassay analyzer the Cobas E801 for high volume laboratories which previously used two E601 analyzers. The E801 utilizes the same electro-chemiluminescence technology, but has 48 reagent slots compared to 25 on the E602. Our laboratory has been using two E601 analyzers since late 2016. We describe our evaluation of the E801 against the E602.

Methods: We evaluated the performance of 14 commonly requested analytes (AFP, CA125, CA15-3, CA19-9, CEA, Cortisol, Ferritin, Folate, NTproBNP, procalcitonin, PTH, testosterone, total PSA, Vit.B12) on the Cobas E801 assays. Evaluation included test imprecision, correlation with the E601, linearity and limits of quantification. Imprecision (coefficient of variation - CV%) was assessed using 2 levels of Roche quality control materials. Statistical analyses were performed on MedCalc software v18.11.3 (MedCalc, Ostend, Belgium).

Results: On the E801, inter-assay CV for various assays at level 1 was <6.5% and <3.5% at level 2. Correlation and regression analyses showed close agreement with the e601 (r >0.99, slope >0.92) for all 14 assays. The limits of quantitation and measuring range were verified. Our verified measuring rage is close to that of the manufacturer's analytical measuring range (AMR) (see Table 1). By replacing two e601 analyzers with one e801 we will save 1.71 square meters of laboratory space.

Conclusion: With a higher throughput (up to 300 tests/hr), smaller footprint, ability to load supplies without interruption of instrument operations, good assay precision, close correlation with the existing E602, and longer calibration curve stability/onboard reagent stability, the E801 is an excellent upgrade from the previous E601.

Table 1. Measuring Range Verification

Analyte	Manufacturer's AMR	Verified Measuring Range
AFP (ng/mL)	2.72 – 1210	0.97 – 1150
CA125 (U/mL)	2.0 – 5000	2.3 – 1078*
CA15-3 (U/mL)	3 – 300	4.25 – 369
CA19-9 (U/mL)	2.7 – 1000	2.1 – 1073
CEA (μg/L)	1.8 – 1000	0.46 – 940
Cortisol (nmol/L)	3.0 – 1750	22.6 – 1639
Ferritin(μg/L)	2.0 – 2000	0.83 – 1921
Folate (nmol/L)	4.54 – 45.4	1.53 – 45.15
NTproBNP (ρg/mL)	50 – 35000	5 – 32709
Procalcitonin (ng/mL)	0.06 – 100	0.02 – 98.3
PTH (ρmol/L)	0.64 – 530	0.38 – 424
Testosterone (nmol/L)	0.4 – 52	1.11 – 46.35
Total-PSA (ng/mL)	0.03 – 100	3.92 – 91.85
Vit. B12 (ρmol/L)	111 – 1476	93.85 – 1514

** Highest concentration tested*