Systemic inflammation affects human osteocyte signaling

L. L. Pathak1, A. D. Bakker2, F. P. Luyten3, P. Verschueren4, W. F. Lems5, J. Klein-Nulend6, N. Bravenboer7, 1School of Pharmaceutical Science and Technology (SPST), Tianjin University, Tianjin, China, 2Department of Oral Cell Biology, Academic Centre for Dentistry Amsterdam (ACTA), University of Amsterdam and VU University Amsterdam, MOVE Research Institute Amsterdam, Amsterdam, Netherlands, 3Skeletal Biology and Engineering Research Centre, KU Leuven, Leuven, Belgium, 4Department of Rheumatology, VU University Medical Center, MOVE Research Institute Amsterdam, Amsterdam, Netherlands, 5Department of Clinical Chemistry, VU University Medical Center, MOVE Research Institute Amsterdam, Amsterdam, Netherlands

Background: Bone remodeling, phosphate homeostasis, and cardiovascular function are disturbed in rheumatoid arthritis (RA), possibly as a result of elevated levels of circulating inflammatory cytokines. Osteocyte signaling plays a vital role in bone remodeling, phosphate homeostasis, and cardiovascular function. Therefore we aimed to investigate the effect of RA-serum or inflammatory cytokines on human osteocyte signaling.

Methods: Human trabecular bone chips containing live osteocytes in their native matrix were cultured with RA-serum or inflammatory cytokines for 7 days. Live-dead staining was performed to assess cell viability. Gene expression of osteocyte signaling proteins and cytokines was analyzed by qPCR. Immuno-staining was performed for osteocyte-specific markers.

Results: Approximately 60% of the osteocytes on the bone chips were alive at day 7. Cells in or on the bone chips did express the gene for osteocyte signaling molecules SOST, FGF23, DMP1, and MEPE, and the cytokines IL-1β, IL-6, and TNFα at day 0 and 7. Treatment with RA-serum, IL-1β, or TNFα enhanced gene expression of IL-1β (8 to 15-fold) and TNFα (2 to 3-fold). Treatment with IL-1β or TNFα, but not RA-serum, also enhanced gene expression of IL-6 (25 to 32-fold) and IL-8 (24 to 58-fold). The stimulatory effect of the combination of IL-1β, TNFα, and IL-6 on gene expression of IL-1β, IL-6, and IL-8 was significantly higher (80 to 120-fold) than the effect of the individual cytokines. IL-1β, TNFα, and the combination of IL-1β, TNFα, and IL-6 enhanced FGF23 expression (2 to 4-fold). SOST expression was enhanced by IL-1β (5-fold), while RA-serum increased both SOST (2.5-fold), and DKK1 (2-fold).

Conclusion: Osteocyte is not just a bone cell, it also has endocrine function. Osteocyte signaling, was affected by RA-serum, individual exogenous recombinant cytokines, and a combination of IL-1β, TNFα, and IL-6 suggesting that osteocytes could provide a new target to prevent inflammation-induced bone loss, disturbed phosphate homeostasis, and cardiovascular diseases.

Information content of paired measurements of A1c and 1,5-anhydroglucitol relative to post-prandial glucose excursions: a model simulation study

J. D. Molligan, L. J. McCloskey, D. F. Stickle, Jefferson University Hospitals, Philadelphia, PA

BACKGROUND: The magnitude of post-prandial glucose excursions (PPGEs) is arguably a factor contributing to diabetes complications beyond its influence on average glucose (G) as evaluated by A1c. 1,5-anhydroglucitol (AG; diet-derived; reference interval: 7-33 μg/mL) is in principle a high-side marker for hyperglycemia, responsive via decreased reabsorption when G exceeds a threshold of approximately 130 mg/dL. However, there are no established guidelines to interpretation of AG measurements. Our objective was to examine the predicted information content of paired A1c and AG measurements with respect to PPGEs. METHODS: We used an established mass balance model for AG (PMID: 9357814), assuming a basis of fixed normal GFR (100 mL/min) and population-average AG ingestion rate (4.6 mg/d). PPGEs were characterized as a fixed waveform (shape) characteristic of diabetes (maximum at 2h; half-maximum at 3h; duration 5h) with variations in specified height (H, maximum mg/dL). PPGEs were added to fasting plasma glucose (FPG) 3 times per day at 0600, 1200 and 1800 hours to form 24-h waveforms, G(t). For a given average H, individual PPGEs were varied according to a normal distribution of H(average) ±20% (1sd). The AG mass balance model was used to simulate changes in AG as a function of FPG and H(average). Simulations (n=100 per condition; 1 min intervals) were conducted until daily average AG was invariant (120 days). Average glucose was calculated from cumulative G(t) to determine associated %A1c. RESULTS: Figure shows simulated model relationship between AG (±1sd; y-axis normalized to baseline AG (H=0)) and H(average), with constant %A1c as a parameter. AG decreased with increased H. Resolution (ΔAG/ΔH) decreased substantially with increasing %A1c, due primarily to decreased baseline AG. CONCLUSIONS: Results provide a resource for interpretation of paired A1c/AG measurements relative to PPGEs. Caveats, however, include restriction of analysis to basis parameters (average AG ingestion rate, normal GFR) and a representative but fixed PPG waveform.
Background: There is much debate regarding osteoporosis in elderly males and its relation to the declining level of Testosterone and Dehydroepiandosterone (DHEA) in males after the age of 60 years which is responsible for fragile fractures. The purpose of this study is to determine the incidence of osteoporosis in males above the age of 60 years and their correlation with Testosterone and DHEA.

Methods: It’s a prospective, cross sectional study in which 100 male patients of 60 years and above were evaluated for Osteoporosis. Patients were selected based on age, clinical signs and symptoms, who had visited as a participant of Orthopaedic camps for the assessment of Osteoporosis by Bone Mineral Density (BMD) machine organized by Universal College of Medical Sciences and Teaching Hospital. BMD were done for all male patients above 60 years at distal radius using quantitative ultrasounds. Blood samples were taken simultaneously after BMD record and sent for determination of Total Testosterone and DHEA level. The Total Testosterone and DHEA were assessed by competitive immunoassay technique using Human ELISA kit, Germany.

Results: Out of enrolled 100 men above 60 years suspected of Osteoporosis, 46 % is diagnosed as Osteoporosis, 32% as Osteopenia and 22% were observed to be normal based on BMD T-Score value. The average level of Serum Total Testosterone and DHEA in entire suspected osteoporosis patients were 2.74 ± 1.04 ng/ml and 1.45 ± 1.08 ng/ml respectively. The level across Osteoporotic cases (n=46), the average Total Testosterone and DHEA were 2.20 ± 0.77 ng/ml and 1.55 ± 0.91 ng/ml respectively. The cut off value by the International Society of Andrology considers abnormally low serum testosterone <3 ng/ml which includes 39 (84%) cases of osteoporotic men compared with normal testosterone level men. Testosterone deficiency was defined as a level of total testosterone <3 ng/ml which includes 39 (84%) cases of osteoporosis. This study has shown that the men with low testosterone levels had decreased BMD T-scores across entire cases but were statistically insignificant. BMI was not significant but inversely associated with testosterone and DHEA levels. In this study male above 60 years old osteoporotic patients with decreased plasma testosterone had a 14 fold higher risk for decreased BMD compared with their peer with normal testosterone level. The Correlation analysis of testosterone levels has shown significant association with BMD (r=0.57, <0.001) and BMD with age (r=-0.24, <0.01) respectively.

Conclusion: In this study, the incidence of osteoporosis increases with advancing age. It can be concluded from the statistical evidence that Testosterone play an important role in the progress and maintenance of osteoporosis but DHEA shows no such evidence in osteoporosis. But still further study is needed to clearly identify the role of testosterone in osteoporosis with interventional approaches in a large population. There was significant decline in Testosterone and DHEA with advancing age and moreover decreased in BMD indicating the pathogenesis of osteoporosis in the patients.

Methods: We did a preliminary evaluation for the salivary cortisol determination in our laboratory using the LIAISON® Cortisol assay (DiaSorin S.p.A., Saluggia (VC), Italy), validated by the producer for serum, plasma and urine samples. To fulfill the validation procedure we followed also the CLSI C49-A guideline (Analysis of Body fluids in Clinical Chemistry: Approved Guideline) measuring cortisol in 44 saliva samples, collected from apparently healthy donors between 11 pm - 12 pm (n= 25) and 8 am - 9 am (n= 19). The same samples have been measured with another method validated from the producer for saliva samples: Cortisol II on Eleyecs® 2010 (Roche, Mannheim - Germany). The samples have been collected by spontaneous salivation with the Salivate® device (Sarstedt, Nürmbrecht - Germany) and following centrifugation with no further treatment analyzed. The morning untreated samples which cortisol concentrations were detectable by both methods, underwent also measurement after extraction with dichlormethane, evaporation and resuspension in an adequate matrix for immunooassay chemical.

Results: Measured concentrations ranged from <0.054 to 1.19 mg/dL and from 0.16 to 3.18 mg/dL with both Eleyecs® e LIAISON® XL systems, respectively. Comparing the results from the two analytical methods, a high correlation is evident (R²=0.973, P<0,0001), both for direct measurement and with extractive methodology, but the regression equation of Passing & Bablok (y= 2,14x + 0,07) shows a significant difference between the methods, proportional to the measured sample concentration, even if the intercept is negligible. Regarding the nocturnal collected samples, whose concentration were undetectable by the Eleyecs® system (< 0.054 mg/dL), meanwhile with the LIAISON® XL system the concentrations ranged between 0,16 and 0,29 mg/ dL. The imprecision of the measured samples by the LIAISON® XL system resulted below 6%.

Conclusion: From the obtained results of measured salivary samples by the LIAISON® Cortisol assay on the LIAISON® XL system we can conclude that further investigations are needed. Both the analytical and the reference ranges should be defined compared to the clinical status of patients.

Correlation of Thyroid function and biochemical parameters in type II diabetic subjects of Western Nepalese population

S. K. Jha1, N. K. Yadav1, U. Jha2, P. K. GS3, D. R. Pokharel1, M. Sigdel4, P. S. Shukla1, 1Manipal College of Medical Sciences, Pokhara, Nepal, 2Pokhara University, Lekhnath, Kaski, Nepal

Background: The prevalence of type II DM is continuously increasing worldwide. These disorders are shown to be associated with, among many other abnormalities and with thyroid dysfunctions. However these association have so far not been explored among Nepalese type II DM. Main aim of the present study was done to evaluate thyroid function in type II diabetics.

Methods: It was a hospital based case control study including 100 type II DM patients and 100 control. All subjects having no any known thyroid and other chronic illness. All the demographic and anthropometric data were collected using a preformed set of questionnaire and biochemical data were obtained from the laboratory analysis of the patients’ blood samples. Statistical analysis was done with SPSS version 17.
Results: In this study we found overall of 17% prevalence in DM cases with thyroid disorders and amongst this, 11.76% with Primary Hypothyroidism, 76.48% with Sub clinical Hypothyroidism and 11.76% with Sub clinical hyperthyroidism. A statistical significant difference was noted between cases-DM and controls with respect to BMI (p<0.000), arm circumference (p<0.000), FT3 (p<0.004), TSH (p<0.000), FBS (p<0.000), PP (p<0.000), HaA1C (p<0.000), TC (p<0.000), TG(p<0.005) and LDL (p<0.018) respectively. In this study, the mean±SD of FT3, FT4 and TSH in control and DM were found to be (2.43±0.64 and 2.67±0.93 with p-value 0.004), (1.06±0.27 and 1.15±0.31 with p-value 0.31) and (2.62±1.42 and 3.70±5.13 with p-value 0.00). Analysis between serum FT3, FT4 and TSH with respect to baseline characteristics and biochemical parameter of the study subjects showed negative significant correlation (p<0.05) between FT4 with region in DM, positive significant correlation between FT4 with Age in DM, positive significant correlation (p<0.05) between TSH with TC in DM. 

Conclusion: This study confirms that thyroid dysfunctions is also common among Nepalese type II DM patients. Our study also reveal that prevalence of thyroid dysfunction is more common in type II DM. It is thus recommended that these group of population should be routinely screened for asymptomatic thyroid dysfunctions besides their usual treatment.

Graves disease: Patients with hyperthyroid status have a higher risk of developing type 2 diabetes

M. RIBERI1, M. Gaston2, A. Castillo3, 1LABORATORIO CASTILLO-CHIDIAK, CORDOBA, Argentina, 2Departamento de Quimica, Facultad de Ciencias Exactas, Fisicas y Naturales, Universidad Nacional de Cordoba, CORDOBA, Argentina

Background. Graves’ disease (GD) is a multi-systemic autoimmune disorder caused by thyroid stimulating antibodies that bind to and activate the thyroid stimulating hormone (TSH) receptor on thyroid cells (TRAbs). Common findings are low serum concentration of TSH, positive TRAbs, and high concentrations of anti-thyroid peroxidase antibodies (ATPO). In insulin-dependent diabetics, hyperthyroidism may aggravate glucose intolerance by multiple mechanisms, decreasing responsiveness to insulin. An association between type 1 diabetes mellitus (DM) and autoimmune reaction to thyroid antigens, including anti-thyroid antibodies (ATPO) in pediatric patients with positive TRAbs, was recently reported. The objective of this study is to investigate the association between thyroid status, serum TSH levels, positive TRAbs and ATPO, and the potential risk to develop type 2 DM based on insulin levels in adults. Methods. The study was conducted in 64 patients between May 2014 and October 2015. The mean subject age was 47 ± 8 years old and the male:female ratio was 11 (17.7%) male:51 (82.3%) female. Pregnant women and patients under 25 years of age were excluded. We measured TRAbs, ATPO, TSH, and insulin concentrations in euthyroids (TSH = 1.10 to 9.00 µU/mL) and hyperthyroids (TSH between 0.01 to 0.44 µU/mL). TRAbs were measured by second generation thyrotropin-binding inhibitor immunoglobulin (TBI) assay (DiaMetra, Italy). The cut-off for positive TRAbs was 1.50 U/L. ATPO, TSH and insulin concentrations were determined by chemiluminescent microparticle immunoassay (CMIA) using a Advia Centaur (Siemens, USA). The cut off for positive ATPO was 37 U/mL, reference interval for TSH was 0.4 to 4.4 µU/mL and for insulin was 5 to 20 µU/mL. Data obtained for all measurements of TRAbs, ATPO, TSH and insulin in both groups was analyzed using the Student’s t-test. A p value &lt 0.05 represented a significant difference. Data was expressed as mean ± standard error of the mean (SEM). Results. As expected, TSH serum concentrations were significantly decreased in hyperthyroid patients (0.13 ± 0.03) compared with euthyroid patients (3.31 ± 0.48) (t=12.79; p &lt 0.05). We observed a significant increase in TRAbs levels in hyperthyroid patients (7.67 ± 1.91) compared with euthyroid patients (2.23 ± 0.40) (t=2.07; p &lt 0.05). In addition, we reported a significant enhancement on ATPO levels in hyperthyroid patients (650.8 ± 84.82) versus euthyroid patients (296.2 ± 85.30) (t=3.03; p &lt 0.05). Similarly, higher insulin levels were observed in hyperthyroid patients (15.35 ± 1.94) versus euthyroid patients (9.94 ± 1.43) (t=2.51; p &lt 0.05). Conclusions. Based on the results of the present study we conclude that thyroid autoimmunity is associated with female gender, the presence of anti-thyroid and TSH receptor antibodies, and low levels of TSH. Importantly, higher mean insulin concentrations were observed in hyperthyroid patients. The presence of TRAbs and high insulin concentrations in patients with TSH between 0.01-0.44 µU/mL and positive ATPO, may indicate a higher risk of developing type 2 Diabetes Mellitus in adults. We recommend evaluation of TRAbs and insulin levels in at-risk populations.
Results
The mean serum concentration of adiponectin in women were 5.93±1.9 µg/mL. In linear regression analysis, significant correlations of serum adiponectin were serum insulin (r = -0.439, p=0.000) and insulin resistance (r = -0.415, p<0.001). BMI, waist circumference, systolic and diastolic blood pressure, serum triacylglycerols and low-density lipoprotein (LDL) had negative correlations with adiponectin but statistically not significant (p>0.05). High-density lipoprotein (HDL) correlated positively with adiponectin level (p=0.03). Across quartiles of WC, insulin-resistant (HOMA-IR>2.5) subjects had significantly lower (p=0.05) adiponectin levels when compared with insulin-sensitive (HOMA-IR < 2.5) regardless of the level of abdominal adiposity.

Conclusion
High adiponectin levels are associated with insulin sensitivity and a favourable lipid profile. Serum adiponectin levels are more tightly linked with insulin resistance than with abdominal obesity.

Materials and Methods: A new glycated serum protein assay on Siemens Vista analyzer

Introduction: Glycated Serum Protein (GSP) or fructosamine, estimates the average blood glucose over a 2-3 week period versus over a 3-4 months period for HbA1c. GSP may be used to monitor diabetics with hemoglobinopathies or have conditions that affect RBC (red blood cell) lifespan. HbA1c is falsely decreased when the RBC lifespan is less than 120 days, while GSP is not affected. Fructosamine assay is widely used as an alternate test for certain diabetes patients with hemoglobinopathies and for pregnant woman. However, most of the fructosamine assays that are currently in the market are nitro blue tetrazolium (NBT) based colorimetric assays and they suffer from a variety of interferences like vit-c, bilirubin, glutathione which lead to inaccurate results. These analytical issues led us to investigate an alternate assay that could be adapted to our existing Siemens Vista analyzer.

Study Objectives: The objective of this study is to evaluate and validate a user-defined application protocol for glycated serum protein (GSP) assay from Stanbio Laboratory - an EKF Diagnostics company on Siemens Vista chemistry analyzer. In addition to the method validation, we also established the specimen stability and adult reference ranges for GSP. Materials and Methods: GSP from Stanbio Laboratory - an EKF Diagnostics Company is a new FDA cleared three step enzymatic colorimetric assay based on trinder endpoint reaction measured at 546-600 nm for quantifying GSP. Results and Discussion: The assay was evaluated on Vista chemistry analyzer using open channel user defined method. Performance of the assay was evaluated for inter and intra assay precision, accuracy, linearity, reference ranges and specimen stability.

Results and Discussion: With in-run imprecision was 6.5% for control 1 (mean=264 µmol/L) and 3.7% for control 2 (mean=715 µmol/L). Between-run precision with 17 days were 4.2% (mean=267 µmol/L) and 2.5% (mean = 728umol/L). Analytical measurement range was verified using 5 level calibrators and acceptable across the range (40-1185 µmol/L). Accuracy and recovery of the assay was acceptable with a mean recovery of 100±5% across the analytical measurement range (AMR). All values were considered acceptable. Comparisons between laboratory assay and vendor predicted assay on Stanbio Sirius clinical chemistry analyzer compared well (r2=square=0.996, slope=1.0 and intercept=1.49). Stability studies proved that samples stored at 2-4°C are stable up to 7 days with no significant variations. Lab also verified the reference interval as 151-300 µmol/L using adult patient population (18-65 yrs).

Conclusion: The user defined application for GSP assay enhances the versatility of the Vista system for specialized glycemic monitoring for a specific diabetic subpopulations where the patient has either a genetic variant of hemoglobin (hemoglobinopathy) or a condition or treatment that affects RBC turnover. Furthermore, this application provides laboratories with a simple, sensitive, fast, and convenient alternative glycemic monitoring test with no endogenous substance interference that are typically observed in NBT based colorimetric fructosamine assays.

### Table A-170

<table>
<thead>
<tr>
<th>WC&lt;89 cm</th>
<th>WC 89-96 cm</th>
<th>WC 97-102 cm</th>
<th>WC &gt;102 cm</th>
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<tbody>
<tr>
<td>IR&lt;2.5</td>
<td>IR&gt;2.5</td>
<td>IR&lt;2.5</td>
<td>IR&gt;2.5</td>
</tr>
<tr>
<td>Mean Adiponectin ±SD</td>
<td>6.1±1.49</td>
<td>5.6±1.94</td>
<td>7.27±1.36</td>
</tr>
<tr>
<td>p value</td>
<td>0.046*</td>
<td>0.03*</td>
<td>0.045*</td>
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Difference among WC quartiles by one-way ANOVA: IR<2.5 groups, p = 0.65, IR>2.5 groups, p = 0.32

### Table A-172

<table>
<thead>
<tr>
<th>FT4 (µIU/mL)</th>
<th>FT3 (nmol/L)</th>
<th>FT3 (nmol/L)</th>
</tr>
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<tbody>
<tr>
<td>0.3-5.0</td>
<td>0.3-5.0</td>
<td>0.3-5.0</td>
</tr>
<tr>
<td>0.4-4.0</td>
<td>0.7-4.0</td>
<td>0.7-4.0</td>
</tr>
<tr>
<td>0.5-5.0</td>
<td>1.0-5.0</td>
<td>1.0-5.0</td>
</tr>
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Background:
Second trimester maternal thyroid deficiency has been associated with adverse neurological development in children and a high rate of subsequent permanent hypothyroidism in the mother (1). Accurate assessment of thyroid hormone concentrations during pregnancy is therefore essential. In pregnancy, measurement of free thyroxine (FT4) and free triiodothyronine (FT3) is complicated by increased binding protein levels. Ultrafiltration or equilibrium dialysis followed by tandem mass spectrometry (MS) is a recommended method for improved sensitivity of FT4 concentrations; however, these techniques are expensive and laborious. The present study compares multiple immunoassay methods for FT4 and FT3 with MS to determine suitability of automated assays for large population-based studies in pregnancy. Previously, MS results for FT4 and FT3 have been compared to a limited number of immunoassay methods.

Methods: Residual sera (n=60) for the comparative study were collected, aliquoted, and distributed by the Women and Infants (WIH) laboratory; TSH concentrations were within the reference interval (0.3-5.0 µU/mL) in 50 samples, elevated in 8 samples, and low in 2. Ultrafiltration followed by liquid chromatography-tandem mass spectrometry was performed as previously described (2). Immunoassay platforms for FT4 and FT3 testing included the Abbott Architect i2000sr, Roche cobas e602, Beckman Coulter DxI, and Siemens Immulite 2000. Formal pairwise method comparisons were performed, after logarithmic transformation. This study was approved by the WIH IRB.

Results: Of the 60 samples, one failed MS quality control for FT4 (hyperthyroid) and 18 for FT3 (14 euthyroid and 4 hypothyroid); 41 samples remained. FT4 correlations between the four immunoassays ranged between 0.82 and 0.93; correlations between MS and the four immunoassays, however, were lower (r values: 0.74, 0.74, 0.66, and 0.71 for Architect, cobas, DxI, and Immulite, respectively). Among the three samples with TSH elevations, all four immunoassays ordered the FT4 results the same as MS. FT3 correlations between the four immunoassays ranged between 0.46 and 0.89; correlations between MS and the immunoassays were low (r values: 0.27, 0.40, 0.37, and 0.18, respectively).

Conclusions: FT4 immunoassay measurements appear to be a reasonable surrogate for MS in pregnant euthyroid patients. Agreements between immunoassays for FT4 are high. MS was usable to reliably determine FT3 in 18 pregnancy samples, and agreement between the remaining 41 FT3 MS results with immunoassays was poor. Agreement was also poor between FT3 immunoassays. These results generate concern regarding the reliability and usefulness of FT3 assays in samples from pregnancy. The measurement of total T3 as an alternative to FT3 is currently under investigation.
of repeat testing < 60 d were: increasing age, surgical (vs. medical) discipline and higher HbA1c. For repeats < 7 days, surgical discipline and higher HbA1c were the only significant predictors while for < 3 days, higher HbA1c was the sole predictor.

**Conclusion:** Inappropriate repeats earlier than 60 days represent 2.4% of all HbA1c measurements on inpatients or 332 tests per year. The strongest predictors of early repeat testing are increased HbA1c and surgical patients. Poor clinician understanding of the timeframe for HbA1c change may contribute to this practice - better education and/or introduction of computerized minimum retest interval guidelines should reduce such over-requesting.

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**A-175**

The development of a method for detecting IGF-I misuse in elite athletes

N. Guha, Y. Dennis, W. Boehning, C. Bartlett, D. A. Cowan, P. H. Sønksen, D. Boehning, R. I. G. Holt, 1, 2, 3, 4. Oxford University Hospitals NHS Foundation Trust, Oxford, United Kingdom, 1University of Oxford, Oxford, United Kingdom, 2University of Southampton, Southampton, United Kingdom, 3King’s College London, London, United Kingdom

**Background:** Growth hormone (GH) and insulin-like growth factor-I (IGF-I) have anabolic and metabolic effects that make them attractive as performance-enhancing drugs. Both substances are included in the World Anti-Doping Agency (WADA) list of prohibited substances because of their potential to improve performance and the risks of harm to the athlete’s health. The GH-2000 and GH-2004 research teams developed a method to detect GH misuse based on changes in GH-sensitive serum peptides - the GH-2000 biomarker method. As GH misuse detection methods have improved, athletes may have turned to IGF-I as an additional or alternative doping agent. Recombinant human IGF-I (rhIGF-I) is structurally identical to endogenous IGF-I and the main challenge in detecting rhIGF-I misuse is to distinguish exogenous from endogenous IGF-I. We previously showed that serum IGF-I increases in recreational athletes after administration of rhIGF-I/rhIGF binding protein-3 (rhIGFBP-3) complex for 28 days.

**Objective:** To assess whether measuring other GH-sensitive serum markers, in addition to IGF-I, could improve the sensitivity and specificity of a test for detecting IGF-I misuse.

**Methods:** Serum samples had been stored from a randomised, double-blind, placebo-controlled rhIGF-I/rhIGFBP-3 administration study. 36 recreational athletes (30 men, 26 women, age 18-30 years) were randomly assigned to receive placebo, low dose (30 mg/day) or high dose (60 mg/day) rhIGF-I/rhIGFBP-3 complex. Treatment was self-administered by subcutaneous injection for 28 days. The following serum peptides were measured during the four-week treatment and eight-week washout period, using commercial immunoassays: IGF-II, IGFBP-2, IGFBP-3, acetyl-lactate subunit (ALS), osteocalcin, procollagen type 1 carboxyterminal propeptide (PICP) and type 1 collagen cross-linked carboxy-terminal telopeptide (ICTP).

On each visit day, marker concentrations were compared between treatment groups using one-way ANOVA. Logistic regression was used to determine which combinations of markers could discriminate between treatment and placebo groups, creating four “IGF score” formulae. Useful markers were then used to determine if serum samples collected from 250 elite athletes (161 men, 89 women) and IGF scores were calculated. Decision limits for each score were estimated using the mean and standard deviation of scores in elite athletes. Values above the decision limit suggest an athlete has misused rhIGF-I/rhIGFBP-3. An age-correction factor was incorporated into the score formulae because all scores decreased significantly with age.

**Results:** IGFBP-2 increased and IGFB-1 decreased in both women and men in response to rhIGF-I/rhIGFBP-3 administration. When these markers were combined with IGF-I results in IGF score formulae, all scores increased rapidly during the first week of drug administration, remained elevated throughout the administration period and then declined. On Day 21 of the administration study, the IGF scores had an estimated sensitivity of 80-94% with specificity of 99.9% (equivalent to the WADA required false-positive rate of 1 in 10,000).

**Conclusions:** Serum IGF-I, IGFB-1 and IGFBP-2 concentrations change in response to rhIGF-I/rhIGFBP-3 administration. These markers have been combined to create IGF score formulae. We have proposed decision limits for the IGF scores, based on marker concentrations in 250 elite athletes, which could be used to detect doping with IGF-I.

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**A-176**

Thyroid autoantibodies in pregnancy: changes across trimesters and association with intrauterine growth restriction in a multi-ethnic population

C. K. M. Ho, E. T. H. Tan, K. H. Tan. KK Women’s and Children’s Hospital, Singapore, Singapore

**Background:** Subclinical thyroid diseases are relatively common in women but the significance of detectable thyroid autoantibodies in pregnant women with no clinical thyroid disease is currently unclear. This study aimed to determine the serum levels of thyroid peroxidase antibody (TPO-Ab) and thyroglobulin antibody (Tg-Ab) across all three trimesters in pregnant women in a multi-ethnic cohort. An association between thyroid autoantibodies in maternal blood and intrauterine growth restriction (IUGR) was also investigated.

**Methods:** 926 women with singleton pregnancies confirmed by ultrasonography at less than 14 weeks of amenorrhea were recruited to this study conducted at a maternity hospital. Exclusion criteria included chronic medical conditions, aneuploidy, fetal anomalies and pregnancies ending in termination, miscarriage or fetal death. Women who were on any thyroid medication or had a history of thyroid disease were also excluded from subsequent analyses.

IUGR was defined by estimated fetal weight or abdominal circumference less than the 5th percentile (adjusted for gender and ethnicity) in the presence of either oligohydramnios or abnormal umbilical artery flow or both after 22 weeks of gestation. Participants attended four separate study visits during pregnancy. Venous blood samples were taken at 9-14 weeks (visit 1), 18-22 weeks (visit 2), 28-32 weeks (visit 3), and 34-39 weeks (visit 4). Serum TPO-Ab and Tg-Ab concentrations were measured using Abbott i2000 immunoassays according to the manufacturer’s protocols.

Statistical analyses (chi-squared and Kruskal-Wallis tests) were performed using the Analyse-it software.

**Results:** Median serum TPO-Ab levels demonstrated a mild decreasing trend with increasing gestational age, and were 0.49, 0.49, 0.48 and 0.45 IU/ml at study visits 1, 2, 3 and 4, respectively (p<0.05). In comparison, median serum Tg-Ab levels decreased from 1.25 IU/ml at visit 1, to 1.09 IU/ml at visit 2, 1.02 IU/ml at visit 3, and 1.00 IU/ml at visit 4 (p<0.0001). The proportion of women tested positive for Tg-Ab (4.11 IU/ml or above) also decreased from 20.2% to 15.3%, 14.4% and 13.6% at study visits 1, 2, 3 and 4, respectively (p<0.05).

Women tested positive for both TPO-Ab and Tg-Ab during study visit 2, 3 or 4 were found to be at increased risk of having babies with IUGR, the odd ratios for IUGR in this study group were 2.1 (95% CI, 1.1-4.0) at visit 2, 2.2 (95% CI, 1.1-4.2) at visit 3, and 2.4 (95% CI, 1.2-4.9) at visit 4, compared to controls.

**Conclusions:** Maternal serum concentrations of TPO-Ab and Tg-Ab decreased with increasing gestational age in this study on women with uncomplicated singleton pregnancies. Positivity for both autoantibodies in the mother during the second or third trimester was associated with an increased risk of IUGR of the fetus.

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**A-177**

Evaluation of TSH, FT4 and FT3 assay using a novel automated analyzer for chemiluminescent enzyme immunoassay (AIA-CL2400).

E. Mori, A. Tobita, H. Shimosaka, S. Okubo, M. Kurano, H. Ikeda, Y. Yatomi. The University of Tokyo Hospital, Tokyo, Japan

**Background:** The measurement for free thyroxine (FT4), free triiodothyronine (FT3), and thyroid stimulating hormone (TSH) requires high sensitivity, accuracy and rapidness in clinical laboratories. AIA-CL2400 is a newly developed analyzer with chemiluminescent enzyme immunoassay technique, which automatically measures TSH with a two-step sandwich enzyme immunoassay and FT4 and FT3 with one-step delayed competitive enzyme immunoassays. The aim of the present study is to perform an analytical validation of TSH, FT4 and FT3 assays using the AIA-CL2400 analyzer.

**Methods:** We investigated the within-run and between-day precision, the analytical sensitivity, and the influences of interfering substances for TSH, FT4 and FT3 assays. We also validated linearity and recovery and determined effective sensitivity for the
Development and Validation of a Dried Blood Spot Method for Leptin

J. A. Maggioere, C. Cruzan, K. Urek, E. Roth, A. Gruszecki, D. Quig. Doctor’s Data, Inc., Saint Charles, IL

Background: Leptin is a hormone produced by adipocytes to provide a satiety (fullness) signal to the hypothalamus. In individuals with obesity, leptin levels may remain elevated in circulation due adipose tissue abundance, and leptin resistance may ensue. Recent interest in the analysis of blood leptin has increased among the medical community related to its association with the development of the metabolic syndrome and pre-diabetes. With increasing awareness of the need to detect metabolic syndrome before progression to adult-onset diabetes, a convenient, precise, and accurate method for leptin testing was sought. Our aim was to develop and validate leptin testing in dried blood spot (DBS) samples. Our laboratory has previously validated blood spot methods for other metabolic markers, namely hemoglobin A1c, high-sensitivity C-reactive protein, and insulin. The addition of leptin to this menu of blood spot analytes is designed to provide additional insight into the contributing factors of compromised metabolic control. Single use and self-retracting bloodletting devices enable the self-collection of capillary blood by lay users. Highly-standardized filter paper collection and transport media are increasingly used for clinical laboratory testing. The employment of sensitive enzyme-linked immunosorbent assays (ELISA) that require sample pre-dilution facilitates the expansion of DBS offerings in clinical laboratories for the reliable analysis of emerging metabolic markers, including leptin.

Methods: Capillary blood samples are collected using Surgisafe™ sterile lancets, and spotted onto PerkinElmer® 226 Spot Saver Cards and permitted to dry. Cards are desiccant packaged and shipped via standard postage to the laboratory. From a 50 µL blood drop, ten 3-millimeter spots are punched using a calibrated hole punch and extracted to 280 µL buffered solution, equivalent to the specified serum dilution of the ELISA kit. Extracts are analyzed via an ELISA microplate assay from Alpco Diagnostics. Results are read from 5-point DBS calibration curves, derived from Alpco-provided calibration standards. Analytical precision, linearity, recovery, trueness, reference interval and stability of DBS leptin were assessed.

Results: The intra-assay and total imprecision coefficients of variation (CV) (n=20) at 8.9 ng/mL were 3.7% and 5.4%, and at 12.4 ng/mL were 3.2% and 6.3%, respectively. Leptin in DBS was confirmed linear between 1.0 - 71.0 ng/mL, with recovery between 8.9 ng/mL were 3.7% and 5.4%, and at 12.4 ng/mL were 3.2% and 6.3%, respectively. The distribution of thyroid hormone concentrations in various thyroid diseases was concordant with the clinical characteristics of each disease and the time course of treatment. The correlation of each hormone supports the existence of the negative feedback between thyroid hormones and TSH.

Conclusion: These results indicated that TSH, FT4 and FT3 assays with the AIA-2400 analyzer measures more rapidly and require a smaller amount of sample compared to the AIA-2000 analyzer. Considering that these assays on the AIA-CL2400 analyzer measures more rapidly and require a smaller amount of sample compared to the AIA-2000 analyzer, the TSH, FT4 and FT3 assays on the AIA-CL2400 analyzer will be useful for clinical diagnosis and provide more efficient routine analytical performance in clinical laboratories. Disclaimer: The performance characteristics presented for TSH, FT4 and FT3 are based on an independent, third party study. They do not represent performance claims made by the manufacturer of these assays. The TSH, FT4 and FT3 assays have not yet been reviewed or cleared to be legally marketed in the United States.
or dementia secondary to other diseases. If multiple reports used the same population, the study with the longest follow-up was included. Reviews, case reports, abstracts and conference proceedings were excluded. Two reviewers independently collected the data and assessed the study quality by the Newcastle Ottawa Scale (NOS). Any disagreement between the two investigators was resolved by consensus or by discussion with a third reviewer.

Results: Two case-control and eight cohort studies published from 2003 to 2015 that evaluated a total of 24721 participants, including 1372 patients with dementia, were finally included. Most studies controlled for some conventional risk factors, including age (n=10), gender (n=10) and thyroid medication (n=7). All the included studies were of high quality by NOS and scored from 7 to 9. The average score was 7.4, and the follow-up duration ranged from 4 to 17 years. The relationships between dementia and the per standard deviation (SD) increment of FT4 (random RR=1.08, 95% CI 1.00-1.17) and TSH (fixed RR=0.91, 95% CI 0.84-0.99) were well established. TSH levels in the low category were associated with an increased risk of dementia (fixed RR=1.55, 95% CI 1.24-1.94). However, the positive association was confined to TSH levels below the normal range (fixed RR=1.68, 95% CI 1.25-2.24), not those in the lower tertile of the normal range (fixed RR=1.39, 95% CI 0.98-1.97). Additionally, dementia was not significantly associated with high TSH levels. In the analysis of three studies focused on men, no positive association between dementia and the low or high categories of TSH were found. Furthermore, all the models showed stability in the sensitivity analysis performed by omitting each study and no significant publication bias were detected by Egger’s test.

Conclusion: This meta-analysis suggests that patients with higher FT4 levels and those with TSH levels below the normal range have an increased risk of dementia. However, a relationship between lower tertile of TSH within the normal range and dementia was not well established, and more studies are urgently needed.

A-181
Novel assay for oxytocin using bioluminescence enzyme immunoassay
K. Karasuwa, Y. Sano, H. Arakawa. Showa Univ., Tokyo, Japan

Background: The neurohypophyseal peptide hormone oxytocin acts in the central nervous system and plays an important role in various complex social behaviors, including affiliation, sexual behavior, social recognition, stress buffering, aggression, and trust. There is increased interest in measuring peripheral oxytocin levels to better understand the role of the peptide in human social behavior. The levels of oxytocin in plasma have been measured by radioimmunoassays and enzyme immunoassays (EIA), but the many heterology. Rabbits were immunized with oxytocin bound through the N-terminus to magnetic particles and reacted overnight at 4 °C. Biotinylated oxytocin was then added and measured up to 1×10^8 mol/assay. Next, seven labeled antigens were used to bridge-link compounds with structures similar to that of oxytocin: AVP, LVP, and VT. Cross-reactivity with all three compounds was less than 0.01%, indicating that this antisera is very highly specific for oxytocin. In addition, there was a good correlation of oxytocin measured values between colorimetric assay and bioluminescent assay (r = 0.9665, n = 48).

Conclusions: The bioluminescent method is sensitive and more specific than conventional immunoassays for oxytocin and can be applied to the determination of plasma oxytocin levels.

A-182
Age and gender related differences in concentrations of parathyroid hormone-related protein measured by LC-MS/MS
M. M. Kushnir1, J. Hunsaker1, J. A. Straseskii2, ‘ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, 2Department of Pathology, University of Utah, Salt Lake City, UT

Background: Measurement of parathyroid hormone related protein (PThP) is diagnostically useful in patients suspected of hypercalcemia of malignancy. PThP and its gene are also known to be expressed in a number of normal cells and tissues. We previously developed a method for the measurement of PThP in plasma using LC-MS/MS and established reference intervals in healthy adults. Measurable concentrations of PThP were observed in plasma samples of all participants, suggesting that PThP is present in circulation in health. The aim of this study was to evaluate associations between PThP concentrations and age in men and women and to evaluate between-gender differences. Methods: The LC-MS/MS method was fully validated according to CLSI guidelines and is in routine use in a clinical laboratory. The analysis was performed as follows, stable isotope-labeled internal standard was added to samples and PThP was enriched using anti-PThP antibody conjugated to magnetic beads, digested with trypsin and samples were analyzed by LC-MS/MS. The lower limit of quantification and upper limit of linearity of the assay were 0.3 and 1100 pmol/L, respectively. Total imprecision of the method was <10%. Specificity of the measurements was confirmed by monitoring two mass transitions of PThP and the internal standard. Using this method we analyzed 284 plasma samples collected from adults: 132 men (age 18-81 y, mean 40 y) and 152 women (age 18-84 y, mean 41 y). Differences between groups were evaluated using nonparametric statistics; p-values ≤0.05 were considered as statistically significant. Results: Overall, significantly higher PThP concentrations were observed in women compared to men (p<0.0001). In women, the highest concentrations were observed in the 21-30 y group; conversely, the lowest concentrations were the lowest in the 41-50 y group and were progressively higher in the groups of older women. Statistically significant differences in concentration were observed between the age groups of women 18-30 y and 31-40 y (p=0.0078); 18-40 y and 41-50 y (p<0.0045); and 41-50 y and 51-84 y (p=0.0001). Statistically significant higher concentrations were observed in women of post-menopausal age (>50 y vs. <50 y, p=0.0009). In men, the lowest concentrations were observed between the ages of 21 and 50 y. In men, significantly lower concentrations were observed in the age group 51-60 y, as compared to 61-81 y (p=0.019). Statistically significantly higher concentrations were observed in women than in men in the age groups of 21-30 y (p=0.0017), and 51-84 y (p=0.0054). Conclusion: Concentrations of PThP were higher in women than in men and were statistically significantly higher in older individuals in both sexes. The highest concentrations of PThP were observed in women 21-30 y and >51 y, and in men >71 y. This sex and age distribution overlaps with some age-related diseases, including osteoporosis; these observations raise questions regarding PThP’s involvement in disorders of calcium regulation associated with age.

A-183
Development of an Anti-Müllerian Hormone Assay on the ADVIA Centaur XP Immunoassay System
J. Bogdanovic1, T. Chuang1, A. Jacewicz1, T. Tran1, S. Sinha1, A. Han1, M. Shaheen1, W. Bedzyk2, D. Hovanec-Bezrucl1, ‘Siemens Healthcare Diagnostics, Tarrytown, NY, 1Siemens Healthcare Diagnostics, Los Angeles, CA, 2Siemens Healthcare Diagnostics, Newark, DE

Background: Anti-Müllerian hormone (AMH), or Müllerian-inhibiting substance, is a transforming growth factor beta protein that controls proliferation and differentiation of the pre-implantation female sex cells. AMH is produced by the Sertoli cells in the fetal testis and is a key factor in the regression of the Müllerian ducts in the male fetus. AMH is a marker of ovarian reserve and serves as a guide in fertility treatments.
in various cell types, including embryonic and adult tissues. AMH levels vary with gender and age but are also influenced by other biological fluctuations. In published clinical studies, in-vitro AMH levels are known to correlate with the antral follicle count for assessment of the ovarian reserve and the onset of menopause. Abnormal AMH values are also known to be associated with polycystic ovarian syndrome and existence of specific tumors. The objective of this study was to evaluate the initial analytical characteristics of an AMH assay from Siemens Healthcare Diagnostics on the ADVIA CentaurXP Immunoassay System. Methods: A single-pass sandwich immunoassay for the detection of AMH has been developed using direct chemiluminescent technology, which uses two antibodies to AMH. The first antibody in the site reagent is a mouse monoclonal anti-AMH antibody labeled with acridinium ester. The second antibody in the solid phase is a biotinylated mouse monoclonal anti-AMH antibody, which is coupled to streptavidin coated magnetic particles. A direct relationship exists between the amount of AMH present in the patient sample and the amount of relative light units (RLUs) detected by the system. Method comparison (using 45 female and 5 male samples; age: 1 month to 53 years), precision (three levels with means of 0.55, 5.5, 16.4 ng/mL), and linearity studies were performed using the ADVIA Centaur XP system. Stability of the native purified AMH was also evaluated up to 51 days at 2-8°C. Results: Method comparison between the ADVIA Centaur XP AMH assay and the predicate assay showed a Passing-Bablok regression slope of 1.00 (dose range from 0.10 ng/mL to 22.4 ng/mL; n = 44) and slope of 1.02 (dose range from 0.10 ng/mL to 112 ng/mL; n = 47; three samples were excluded due to the dose being below detection limit in both assays). Repeatability coefficients of variance (CV) for three samples at the concentrations stated above were 3.7%, 2.1%, and 2.5%, while within- and precision CVs were 8.0%, 3.8%, and 4.9%. Assay linearity was demonstrated from 0.10 ng/mL to 67 ng/mL (linear regression slope: 0.95; 95% confidence interval: 0.92-0.99). The stability study for the purified AMH after storage in protein buffer at 2-8°C showed average dose recovery of 99% as compared to the AMH concentrations measured on Day 0 (0.30±0.58 ng/mL). Conclusion: The results from the studies have demonstrated reproducible and scalable performance that is also comparable to a widely used method. Stability of the native purified AMH will improve the performance of the assay with enhanced in-use and storage life of the calibrator and quality control materials. *Disclaimer: Under feasibility evaluation. Not available for sale and its future availability cannot be guaranteed.

A-184 Analytical Performance Characteristics of the New Beckman Coulter Access TSH (3rd IS) Assay


Background: Beckman Coulter has developed a new highly sensitive 3rd generation TSH assay for use on the Access Immunoassay Systems called the Access TSH (3rd IS) assay. TSH (thyroid-stimulating hormone) is the principal regulator of thyroid function, stimulating the synthesis and release of thyroid hormones thyroxine (T4) and triiodothyronine (T3). The principal clinical use for TSH measurement is for the assessment of thyroid status.

Methods: Beckman Coulter Access TSH (3rd IS) assay is a paramagnetic particle, chemiluminescent sandwich immunoassay for the quantitative determination of thyroid-stimulating hormone in human serum and lithium heparin plasma. Two mouse monoclonal antibodies are utilized in the sandwich assay, one as an anti-TSH alkaline phosphatase conjugate and the other is immobilized on paramagnetic mouse monoclonal antibodies are utilized in the sandwich assay, one as an anti-TSH alkaline phosphatase conjugate and the other is immobilized on paramagnetic particles.

Results: The Access TSH (3rd IS) assay demonstrated acceptable linearity throughout the analytical measuring range of 0.005 to approximately 50.0 µIU/ml, and is capable of measuring samples above the range using sample dilution with recovery of ≥ 93%. The LoB for the TSH (3rd IS) assay was measured at 0.0004 µIU/mL, the LoD was measured at 0.0008 µIU/mL, and the LoQ was measured at 0.0013 µIU/mL. Four samples ranging from concentrations of 0.02 - 38.76 µIU/mL were used to test for assay imprecision over 20 days. Within-run imprecision was 2 - 4% and total imprecision was 3 - 6% for the samples. No cross reactivity was observed to hCG at 1,000,000 µIU/mL (< 0.01%), to hFSH at 1,000 µIU/mL (< 0.1%), or to LH at 3,000 µIU/mL (< 0.1%). No interference was detected from endogenous preanalytical interferences. Interference with albumin (450 µg/mL), hemoglobin (10 mg/mL), or triglycerides/Lipid (33 mg/mL) was also comparable to a widely used method. Stability of the native purified AMH will improve the performance of the assay with enhanced in-use and storage life of the calibrator and quality control materials.

Conclusions: The new Beckman Coulter Access TSH (3rd IS) assay is a 3rd generation TSH assay capable of TSH measurement in hyperthyroid patient samples down to 0.001 µIU/mL with the accuracy and precision expected from a 3rd generation TSH assay.
Assessment of Gender-Related Differences in Vitamin D levels, Cardiovascular Risk factors in Saudi Patients with Diabetes Mellitus

A. ALJOH1, M. Abudawood2, K. Almousa1, S. Sobki1, S. Ansar2, 1Prince Sultan Military Medical City, Riyadh, Saudi Arabia, 2King Saud University, Riyadh, Saudi Arabia

Diabetes is a major risk factor for cardiovascular disease (CVD) including coronary heart disease, stroke and peripheral artery disease. CVD remains a leading cause of mortality throughout the world, affecting both women and men. This study aimed to assess gender based differences in cardiovascular risk factors among adults with diabetes mellitus (T2DM). This hospital-based cross-sectional study involving subjects was divided into two gender based groups; male diabetic (n=800) and non-diabetic (n=800) and female diabetic (n=800) and non-diabetic (n=800) for each comparison. Blood samples were analyzed for fasting glucose (Fg), HbA1c, total cho-sterol (Tc), triglycerides (Tg), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and serum levels of 25(OH)-vitamin D in all groups. All the glycomic control parameters, lipid profile parameters were found significantly different in diabetic vs non-diabetic group (p<0.0001) in both genders. Triglyceride (Tg) level was borderline high in T2DM patients in both genders. However, HDL-C levels were significantly lower in diabetic group as compared to non diabetic group in both genders. The results also show that vitamin D concentration was lower in diabetic patients than the healthy individuals. Although the mean concentration of vitamin D in males in both groups was equal but in the women with diabetes was lower than the healthy women. Routine screening of these parameters in T2DM patients may assist early detection of these parameters and prevent risk of CVD. Women with diabetes mellitus are at higher risk of cardiovascular disease compared to men and this may be a result of greater clustering of risk factors among women. This indicates overall poor risk factor control but worse among women, emphasizing the need for better implementation of guideline recommendations for management of diabetes to reduce future cardiovascular diseases.

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Testing for TSH receptor stimulating immunoglobulins: performances of a novel fully automated assay with improved specificity.

d. GRUSON, B. Ferracin. Cliniques Universitaires St Luc, bruxelles, Belgium

Background: Measurement of TSH receptor autoantibodies (TRAb) is important for the diagnosis and monitoring of Graves’ disease (GD). Several automated methods for testing are available but are not yet standardized and not specific of TSH receptor stimulating immunoglobulin (TSI). Our objective was to determine the performances of a novel automated assay with improved specificity for TSI. Methods: We evaluated the IMMULITE® TSI assay (Siemens), a fully automated immunoassay based on the chimeric receptor that specifically binds TSI but not blocking autoantibodies. Assay’s imprecision was assessed with five pools of serum samples and with two levels of control materials. The linearity of the assay was tested a eight points dilution test of high titer samples. Reference values were determined with samples from 90 male healthy volunteers free of thyroid diseases and medications. Method comparison was performed with a second-generation TRAb enzyme immunoassay Medizym® as well as with the Cobas® 8000 and Kryptor® TRAB automated immunoassays. Results: Between coefficients of variation were 6.5 and 4.7% for concentrations of 1.0 and 22.6 IU/L, respectively. The limit of quantification of the IMMULITE assay, determined with the precision profile built with the 5 pools of serum, was below 0.1 IU/L. The dilution test covered a range of concentrations ranging from 40 to 0.32 IU/L and the mean recovery was 108 %, confirming assay’s linearity. The concordance correlation coefficients between the TSI and TRAb assays were 0.82, 0.68 and 0.82 with Medizym®, Cobas® and Kryptor® methods, respectively. The TSI levels measured with the IMMULITE assay in healthy volunteers were below 0.10 IU/L. The receiving operator curve analysis of patients with active Graves disease patients with other thyroid disorders and healthy controls revealed an AUC of 0.99 resulting in a sensitivity of 100% and a specificity of 99% at a TRAb level of 0.40 IU/L.

Conclusions: Our data showed excellent analytical and clinical performances for this novel fully automated assay with an improved specificity for stimulating antibodies.

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Evaluation of HbA1c turbidimetric immunoassay on the new high-throughput analyzer cobas c513 against ion exchange HPLC method and assessment of HbF interference on HbA1c measurement

P. Reimundo1, H. Valbuena2, S. Bergamo1, I. Comas1, R. Martinez-Lopez2, R. Ferrer2, D. Beneitez2, E. Casis4. Service of Clinical Biochemistry, Hospital Universitari Vall d’Hebron, Barcelona, Spain, 2Clinical Laboratories, Hospital Nostra Senyora de Meritxell, Escaldes-Engordany, Andorra, 3Service of Clinical Analysis, Complejo Hospitalario y Universitario de Albacete, Albacete, Spain, 4Service of Hematology, Hospital Universitari Vall d’Hebron, Barcelona, Spain

Background: diabetes mellitus is a serious lifelong condition characterized by hyperglycemia as a consequence of defective insulin activity. Its prevalence is rapidly increasing worldwide, which turns this disease into a major public health priority. Diabetic patients, and especially those with a poor control of blood glucose levels, are at high risk for subsequent microvascular and macrovascular complications. Glycerated hemoglobin (HbA1c) is a useful diagnostic test for those at risk of suffering diabetes, and the preferred laboratory test to monitor glycemic control in patients treated for diabetes and other glucose-metabolism disorders. There are different methods available for HbA1c measurement, differing in accuracy, specificity and/or processing speed, as well as in the presence/absence of interferences by hemoglobin structural variants, chemically modified hemoglobins or high fetal hemoglobin (HbF) concentrations. More specifically, it has been previously described that anomalous HbF levels (>2%) cause negative interference in HbA1c measurement by turbidimetric immunoassay, but not by ion exchange HPLC, thus obtaining falsely decreased HbA1c results. Objective: the first objective of this study was to compare Tina-quant HbA1c Generation 3 turbidimetric inhibition immunoassay (TIDIA) on the new high-throughput analyzer cobas c513, with an ion exchange HPLC method, widely established in clinical laboratories for measuring HbA1c. The second objective was to determine the interference in HbA1c measurement caused by anomalous HbF concentrations, when using turbidimetric immunoassay on the new cobas c513 analyzer.

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Methods: in order to compare both methods, HbA1c concentrations were analyzed in parallel in 143 whole blood samples on a cobas c513 analyzer and on a Menarini HA-8180V analyzer. Statistical analysis was performed using Pearson’s coefficient and Passing-Bablok regression (MedCalc 12.5 software).

To determine the effect of HbF on HbA1c measurement, HbA1c concentrations were determined in parallel in 50 whole blood samples containing anomalous HbF levels (range 4.2 to 13.5%), again using the turbidimetric immunoassay and the ion exchange HPLC. HbF levels were measured by ion exchange HPLC on a Bio-Rad Variant II analyzer.

Results: a strong positive correlation was observed between Tina-quant HbA1c Generation 3 TINA and the ion exchange HPLC (Spearman r = 0.9964; P < 0.0001; 95% CI r: 0.9949-0.9974). Besides, method comparison demonstrated that both methods are equivalent (Passing-Bablok regression equation: y = 0.01166+1,005556x; 95% CI intercept: -0.00000-0.09308; 95% CI slope: 0.99221-1.0200).

Utilization review of vitamin 1,25 (OH)\textsubscript{D} testing in a large teaching hospital

C. Gavva\textsuperscript{1}, I. A. Hashim\textsuperscript{2}, ‘Department of Pathology, University of Texas Southwestern Medical Center, Dallas, TX, ‘Department of Pathology, Parkland Health and Hospital System and University of Texas Southwestern Medical Center, Dallas, TX.

Background: Consensus opinion supported by recent guidelines \cite{1} recommend against measuring vitamin 25(OH)\textsubscript{D} unless the patient has an acquired vitamin 25(OH)\textsubscript{D} metabolic disorder such as chronic kidney disease, sarcoidosis, and lymphomas or an inherited disorder including congenital rickets and hereditary phosphate-losing conditions. For most other patients, vitamin 25(OH)\textsubscript{D} should be measured to screen for vitamin D deficiency as vitamin 1,25(OH)\textsubscript{D} testing is not yet. Therefore, the specialists of laboratory medicine have to know about the different assay methods and platforms that are currently available in the market. For most other patients, vitamin 25(OH)\textsubscript{D} should be measured to screen for vitamin D deficiency as vitamin 1,25(OH)\textsubscript{D} testing in a large teaching hospital.
testing on a commercially available automated c-peptide test. The sample set included random samples, fasting samples, and post meal samples collected from in house volunteer participants as well as archived samples purchased from a vendor. LoB, LoD, LoQ were evaluated per CLSI EP17-A2 by testing 100 replicates of 1 LoB fluid and 5 LoD fluids over 5 days. High dose hook was assessed up to 2000ng/mL. Testing was conducted across two reagent lots and three VITROS® systems. Results: The within lab %CVs ranged from 2.8% to 3.8% on the VITROS® 5600 and 2.1% to 3.6% on the VITROS® ECI for samples ranging in concentration from 0.28 to 12.4ng/mL. At 1000ng/mL, the observed % cross reactivity for proinsulin was 0.5%. At 26.396µIU/mL of insulin, no cross reactivity was detected. For the method comparison, Deming regression analysis yielded slopes ranging from 0.98 to 1.04, intercepts ranging from -0.02 to 0.07, and Pearson Correlation Coefficients ranging from 0.99 to 0.98 among the VITROS® 5600, VITROS® 3600 and VITROS® ECI systems. The overall mean % bias for the prototype method ranged from 0.56% to 4.25% among the VITROS® 5600, VITROS® 3600 and VITROS® ECI compared to the commercially available automated comparator method. The LoB was 0.090ng/mL, The LoD was 0.027ng/mL, and the LoQ at 20% CV was 0.045ng/mL. No high dose hook was observed for the assay up to 200ng/mL. Conclusion: Preliminary performance data demonstrate that the prototype assay has excellent precision, minimal to no cross reactivity with proinsulin and insulin, excellent correlation with a commercially available method, an LoQ consistent with other commercially available methods, and shows no high dose hook up to 200ng/mL.*Under development

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IMMULITE 2000 IGF-I Assay Restandardization to 1st WHO IS for Insulin-like Growth Factor-I, NIBSC Code 02/254*


Background: The IMMULITE® 2000 IGF-I assay from Siemens Healthcare is a solid-phase, enzyme-labeled, chemiluminescent immunometric method for in vitro diagnostic use with the IMMULITE 2000 Systems Analyzers - for the quantitative measurement of insulin-like growth factor I (IGF-I) in serum or heparinized plasma, as an aid in the evaluation of growth disorders. The IMMULITE 2000 IGF-I assay has been restandardized to the 1st WHO International Standard for IGF-I, NIBSC code 02/254, with full performance verification studies carried out to demonstrate its appropriate analytical performance. Methods: Alignment of the restandardized IMMULITE 2000 IGF-I assay to the WHO standard was demonstrated by accurate recovery of various graviometrically prepared WHO 1st IS 02/254 spiking solutions in native patient samples. A method comparison of 164 native patient sera with IGF-I concentrations ranging from 23.0 to 900.0 ng/mL was conducted between the restandardized IMMULITE 2000 IGF-I assay and the IDS-iSYS IGF-I assay (which closely aligned to the WHO 1st IS 02/254 and compares well with the IDS-iSYS reagent lots.

Comparison studies showed less than 3% variation between three unique verification reagent lots. The restandardized IMMULITE 2000 IGF-I assay and the IDS-iSYS IGF-I assay demonstrated LoQ consistent with other commercially available methods, and shows no high dose hook up to 200ng/mL.

Conclusion: The diagnostic sensitivity of the assay was evaluated and the results were reported in ng/mL (CLSI EP17-A2). Method comparison against the IDS-iSYS and IMMULITE 2000/XPi IGF-I assays, and between ADVIA Centaur XP and CP Systems, were evaluated using 50 apparently healthy patient samples (CLSI EP9-A3). Additional performance studies included linearity (CLSI EP17-A2), cross-reactivity to IGF-1, IGF-II, IGF-BP-1, IGF-BP-2, IGF-BP-3 and IGF-BP-6 (CLSI EP7-A2), and precision using a 20-day protocol on two systems, two runs/day (CLSI EP5-A3). Finally, the stability of the reagents was assessed by measuring on board stability and the calibration interval of the assay (CLSI EP25-A).

Results: The ADVIA Centaur IGF-I assay in development had a LoB/LoD/LoQ of 0.007ng/mL/0.027ng/mL/0.28ng/mL, respectively, on ADVIA Centaur XP with a working range up to 16,000 ng/mL. ADVIA Centaur XP and Centaur CP agreed with a slope of 1.00 and R² = 0.990, whereas comparisons between ADVIA Centaur XP IGF-I and IDS-iSYS and IMMULITE 2000/XPi IGF-I had slopes and R squared values of 1.026 and 0.966, and 1.003 and 0.966, respectively. Between run and between day precision of the ADVIA Centaur IGF-I assay showed <5% CV on both ADVIA Centaur XP and ADVIA Centaur CP Systems for patient samples covering IGF-I concentrations of 800-7,000 ng/mL. The assay demonstrated no cross-reactivity to IGF-I, IGF-II or any of the IGF Binding Proteins, and exhibited excellent linearity from LOQ to 16,000 ng/mL having a weighted linear fit slope of 1.05 and intercept of -1.60. The assay had 35 days on board stability (OBS) and a 35 day calibration interval.

Conclusion: The results of these studies show good performance of the fully automated ADVIA Centaur IGF-I assay and good agreement with the IDS-iSYS and IMMULITE 2000/XPi assays.

* For investigational use only. The performance characteristics of this product have not been established. Not available for sale.

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OPTIMIZING CORTISOL AFTER INSULIN STIMULATION IN CHILDREN: WHEN AND HOW MANY TIMES SHOULD WE COLLECT BLOOD SAMPLES TO ACCURATELY EXCLUDE HYPOCORTISOLISM

Y. Schrank, R. Fontes, G. Rivelli, E. M. Ribeiro, M. D. Freire, O. F. Silva. DASA - Brazil, Rio de Janeiro, Brazil

Background: The insulin tolerance test (ITT) was developed in the late 1960s, and remains as the ‘gold standard’ test to determine the need for cortisol replacement in patients with hypothalamic-pituitary-adrenal disease. It assesses the integrated central and peripheral responses to a stressful event caused by hypoglycemia. However, this test is unpleasant for patients, is resource-consuming, and is not without risk. It has to be performed in a specialized unit with adequate supervision, and patients with documented ischemic heart disease or seizure disorders should not undergo ITT and require alternative diagnostic tests. Despite wide experience, uncertainty remains regarding the optimal duration of the test. Objectives: In order to optimize the specimen collection for the ITT we studied timing of peak cortisol value. The aim of our study was to examine whether the cortisol stimulation test could be performed with fewer samples without compromising its diagnostic value. Methods: We performed a cross-sectional retrospective examination of 297 consecutive children submitted to cortisol stimulation test with insulin who showed a positive response (cortisol ≥ 18 mcg/dL) to the stimulus. Regular insulin was applied intravenously at a dose of 0.075 units/kg body weight. If hypoglycemia was not reached 40 minutes after insulin administration, an additional dose, sufficient to achieve blood
glucose levels below 40 mg/dL was applied. Blood samples for cortisol and glucose determination were taken at 0 time, during hypoglycemia and 30, 60 and 90 minutes after hypoglycemia. Serum cortisol concentration was tested with the Cobas analyzer electrochemiluminescence immunoassay and glucose was tested by an enzymatic method. A test was considered responsive when peak cortisol at any time ≥ 18 mcg/dL.

### Results:
Adequate hypoglycemia (glucose < 40 mg/dL) was achieved in all patients. A second insulin dose to achieve hypoglycemia was necessary in 3% of our series. No significant side effects were recorded. The mean age of our patients was 9.9 ± 3, range 5-16 years, with male:female ratio 2:1. Median cortisol values at 0 time, during hypoglycemia and 30, 60 and 90 minutes after hypoglycemia were respectively 11.2, 13.9, 21, and 14.2 mcg/dL. The majority, 178 (60%) of our patients showed peak cortisol 30 minutes after hypoglycemia, which usually corresponds to 60 minutes after insulin administration. Seventy six (25.6 %) showed peak cortisol 60 minutes after hypoglycemia (which usually corresponds to 90 minutes after insulin administration), however 74% of this patients showed cortisol ≥ 18 mcg/dL at any other collection time before. Twenty (6.7%) of our 297 patients showed cortisol ≥ 18 mcg/dL at this time (60 minutes after hypoglycemia) with no cortisol positive answer at any other time. However half of these patients showed a cortisol level ≥ 17 mcg/dL at any other time before the collection 60 minutes after hypoglycemia. **Conclusion:** As cortisol release happens after hypoglycemia, it seems rational to collect samples in accordance with the recommendation of event and not in pre-established times. Collection of cortisol after ITT can be optimized, without sacrificing sensitivity, by collecting only three specimens for cortisol and glucose determination: time 0, during hypoglycemia and 30 minutes after hypoglycemia.

### A-195
#### A Laboratory Comparison Study of the Roche cobas and Siemens IMMULITE 2000 Adrenocorticotropic Hormone (ACTH) Assays

Z. Shajani-Yi1, S. L. La’ulu1, T. J. Brough1, J. S. Straseski1, M. A. Cervinski1
1. Geisel School of Medicine/Dartmouth-Hitchcock Medical Center, Hanover, NH, 1. ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, 2. Dartmouth-Hitchcock Medical Center, Lebanon, NH, 3. Department of Pathology, University of Utah, Salt Lake City, UT

**Background:** The measurement of plasma adrenocorticotropic hormone (ACTH) concentration is integral to the diagnosis of ACTH-dependent and ACTH-independent Cushings’ Disease and primary, secondary and tertiary adrenal insufficiency. The objective of our study was to validate the Roche cobas ACTH assay including a split-sample comparison to the Siemens IMMULITE 2000 assay.

**Methods:** Precision, linearity and carryover studies on the Roche cobas ACTH assay were performed using a cobas e601 analyzer. A method comparison to the Siemens IMMULITE 2000 ACTH assay included 79 patient samples spanning the linear range of the Roche assay. Data was analyzed using EP Evaluator.

**Results:** The Roche cobas ACTH assay demonstrated acceptable within-day precision using Roche PreciControl QC material with coefficients of variation (CV) of 0.8% and 1.0% at mean ACTH concentrations of 49.9 pg/mL and 755.1 pg/mL, respectively. The day-to-day precision study demonstrated CVs of 2.1% and 1.5% at ACTH concentrations of 37.3 pg/mL and 895.7 pg/mL, respectively. The Roche cobas ACTH assay was verified as linear from 1 to 1500 pg/mL and did not display significant carryover. In an attempt to independently verify the Roche cobas ACTH assay performance and to supplement the two concentrations of Roche PreciControl QC material we assayed the Siemens IMMULITE 2000 QC material on the Roche assay. The Siemens QC material demonstrated day-to-day imprecision of 1.2% and 1.4% with observed means of 10.4 pg/mL and 130.0 pg/mL, respectively. Interestingly, the observed concentrations of the Siemens QC material on the Roche assay was between two and four-fold lower than the Siemens expected range, implying non-commutability of the Siemens QC material on the Roche platform. We also tested the opposite combination; analysis of the Roche cobas IMMULITE QC material using Deming regression analysis yielded the following equation (95% confidence intervals): Roche cobas ACTH = 0.708(0.662-0.754)x Siemens IMMULITE ACTH + 0.689(0.644-0.735), r=0.9606.

**Conclusion:** The Roche cobas ACTH assay demonstrated acceptable precision over a twenty-day period; however, the Roche cobas assay demonstrated a negative proportional bias as compared to the Siemens IMMULITE 2000 ACTH assay. We hypothesize that the inter-assay bias is related to differences in assay design. The Roche assay uses two monoclonal antibodies targeted to epitopes between ACTH amino acids (AA) 9-12 and 36-39, whereas the Siemens assay uses one monoclonal antibody targeted to ACTH AA 18-39 and one polyclonal antibody targeted to ACTH AA 1-24. Due to differences in assay design the Siemens assay may recognize the ACTH degradation product corticotrophin-like intermediary lobe peptide (CLIP) (ACTH AA 18-39) possibly contributing to positive bias relative to the Roche assay. Similarly the Roche assay likely demonstrates negative bias relative to the Siemens assay due to competition between full-length ACTH and CLIP for one of the two antibodies on the Roche assay. We are currently further investigating the cause of this inter-assay bias.
physiological variability (male, female, post-menopausal) and the range of estradiol concentrations (2-4000 pg/mL) on ID-GCMS and Beckman Coulter Access Estradiol Sensitive immunoassay. Correlation was assessed using Pearson correlation. Calibration curve and open vial calibrator stability were assessed according to CLSI EP25-A.

Results: The Access Sensitive Estradiol assay is traceable to JCTLM-approved methods according to ISO 17511, covering a measuring range from 15 to 5000 pg/mL. Within-run and within-laboratory are below 6% and 8%, respectively, for a sample at 30 pg/mL and below 3% and 4%, respectively, for a sample at 200 pg/mL. Limit of Detection (LoD) was 10 pg/mL. Limit of Quantitation (LoQ) was 15 pg/mL. Compared to the ID-GCMS method, the Pearson correlation was above 0.98, the method comparison exhibited a slope within 1.00 +/- 0.05 with an intercept below 5 pg/mL. The calibration curve and open vial calibrator stability are 31 and 90 days, respectively.

Conclusion: The Beckman Coulter Access Sensitive Estradiol assay is accurate and precise down to 15 pg/mL, making it an efficient tool for assessing estradiol status of children, males and monitoring females under antiestrogen or hormonal treatment.

A-200

Performance evaluation of the Lifotronic H9,a new HbA1c analyser

D. Wen, X. Zhang, S. Xu, M. Suo. Zhongshans Hospital Affiliated to Sun Yat-sen University, Zhongshan city, Guangdong Province, China

Background: Hemoglobin A1c(HbA1c) is a powerful tool for both monitoring long-term glycemic control, and to diagnostic diabetes. Ion-exchange HPLC (IE-HPLC) assay has been consolidated as a gold standard to measure HbA1c. This study was aimed to evaluate the analytical performance of a new IE-HPLC analyser to measure HbA1c (Lifotronic H9), and evaluate Lifotronic H9 in comparison to two other widely used HPLC systems (Prinus Ultra2® ion exchange HPLC) and Bio-Rad Variant II turbo2.0® ion exchange HPLC) in patients with normal and abnormal Hb.

Methods: The current study was conducted at the Sun Yat-sen University affiliated Zhongshan hospital laboratory. All studies were evaluated using CLSI guidelines. The precision, accuracy, linearity and interference were evaluated according to CLSI protocols EP5-A2, EP9-A3, EP6-A and EP7-A2 respectively. Measurements of HbA1c by the three methods were made in blood from 124 patients with normal Hb(HbA )and 34 patients with abnormal Hb(Hb E). Primus Ultra2 was used as comparative system, and the other 2 systems were test systems. Comparative analysis and bias evaluation were conducted on the results from three detection systems, Appropriateness of data for linear regression analysis was checked regards CLSI EP9-A3 document, then performed both linear regression and difference plot analyses.

Results: The within-run precision values(CV%) were less than 1.75% and the total imprecision values(CV%) were less than 2.02%. Bias using reference samples from NGSP ranged from -2.78 to 2.63%. The linearity of was excellent in the range between 3.0% and 18.0%. Comparison of both methods against respectively, Primus Ultra2 demonstrated significant correlation (Lifotronic H9: r = 0.997; slope = 0.96; intercept = -0.02; Bio-Rad Variant II turbo2.0: r = 0.998; slope = 0.98; intercept = -0.13). The differences of the 95% confidence interval (95%CI) between the test systems and the comparative system in normal HbA samples and HbE samples were within ±0.70% HbA1c, bias% were less than 6%, (P >0.05). The results showed that the Lifotronic H9 and Bio-Rad Variant II turbo2.0 were not affected by HbE.

Conclusion: The Lifotronic H9 demonstrated high analytical performance similar to previous systems such as Primus Ultra2 and Bio-Rad Variant II turbo2.0 widely used HPLC systems. were not affected by HbE, and is therefore suitable for its utilization in modern clinical laboratories.

A-201

Standardization and Harmonization* of Ortho- Clinical Diagnostics Thyroid Function Tests - VITROS® Immunodiagnostic Products TSH and Free T4 Assays (* In development)

C. Thomas1, P. Hosimer2, M. Rymill3, C. Way1, G. Horton1. 1Ortho Clinical Diagnostics, Pencoed, United Kingdom, 2Ortho Clinical Diagnostics, Rochester, NY

Background: The International Federation of Clinical Chemists (IFCC) has an ongoing program of standardization and harmonization for Thyroid function tests (C-STFT). The project aims to develop reference measurement systems (reference materials/reference methods) to establish traceability of free thyroid hormone and TSH assays. The IFCC intends that; FT4 assays will become traceable to the conventional reference measurement procedure based on equilibrium dialysis isotope dilution-liquid chromatography/tandem mass spectrometry (ED ID-LCMS/MS), TSH assays to the statistically inferred all-procedure trimmed mean (APTM).

Methods: Ortho generated data as part of the IFCC Phase IV Harmonization and Standardization study by testing two panels of samples (90 FT4 & 102 TSH samples), results returned to the C-STFT. The committee provided Ortho with panel member results as determined by ED ID-LCMS/MS for FT4 and the statistically derived APTM values for the TSH panels. Ortho then adjusted the values of their master reference calibrators to achieve closer agreement to these values. To achieve the best possible agreement of the VITROS® TSH assay to the APTM values at doses <0.3mIU/mL Ortho introduced two additional reference standards to their master reference calibrator set.

Results: Prior to the recalibration exercise slopes of 0.69 and 1.05 were obtained for the VITROS® Free T4 and TSH assays respectively. After adjustment of Ortho’s internal reference standards, slopes of 1.02 and 1.00 were obtained for the VITROS® Free T4 and TSH assays.

Conclusion: Agreement of the VITROS® TSH assay (at doses below 0.3mIU/mL) against the IFCC APTM panel was improved by introduction of two additional master reference calibrator levels, and manipulation of the assigned doses. Manipulation of the master reference calibrator values for the VITROS® Free T4 assay can improve the correlation to ED ID-LCMS/MS. However the VITROS® Free T4 assay is a true free hormone assay which is currently well aligned with other immunoassays.
**A-202**

What's your favorite color? A comparison of two albumin dye methods and their influence on calculated free testosterone

S. L. La’ulu1, J. A. Strasak2, ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, 1Department of Pathology, University of Utah, Salt Lake City, UT

**Background:** Over the past several decades, measurement of serum albumin has been dominated by two dye methods: bromocresol green (BCG) and bromocresol purple (BCP). Both methods have reported advantages and disadvantages. Even though specificity of BCG has improved since its inception, BCP continues to be more specific. However, BCP methods underestimate serum albumin covalently bound to bilirubin and in dialysis patients. Undoubtedly, the clinical utility of serum albumin and the patient population of the laboratory should be considered. Here we investigated the differences between serum albumin measured using BCG and BCP dye methods by two chemistry analyzers. Furthermore, the influence of albumin on the determination of free testosterone (FT) was assessed.

**Methods:** Residual serum samples from men (n=150), women (n=100), boys (n=25), and girls (n=25) were obtained after completion of clinical testing for total testosterone by liquid chromatography tandem mass spectrometry (LC-MS/MS).

FT was determined in men using equilibrium dialysis (ED)-LC-MS/MS (mean FT concentration, 73.4 pg/mL). All samples were further tested for albumin with both the BCG and BCP dye methods using the Abbott ARCHITECT c8200 and Roche cobas c702. FT was calculated using the Veermale equation, where total testosterone determined by LC/MS/MS and sex hormone binding globulin measured by Roche Modular E170 were used (mean calculated FT concentrations ranged from 71.3-73.7 pg/mL). The following comparisons were made: 1) albumin comparisons between the two dyes (BCG and BCP) for each analyzer, along with comparing the same dye between the ARCHITECT and c702, 2) comparisons of calculated FT using different albums, and 3) comparison of calculated FT with directly measured FT in men; including calculated FT using a generic albumin value of 4.3 g/dL. Significance was characterized as *p*-values ≤0.05, determined by paired t-tests.

**Results:** For all populations combined, serum albumin mean concentrations were 4.4, 4.3, 4.2, and 4.1 g/dL for the ARCHITECT BCG, ARCHITECT BCP, c702 BCG and c702 BCP, respectively. When comparing these 4 datasets against each other, the associations were all significantly different (*p*-values ≤0.0001). To evaluate whether or not these significant differences in albumin influenced calculation of FT, each albumin dataset was entered into the Veermale equation, and all other variables remained the same. Comparisons of calculated FT were all significantly different (*p*-values ≤0.0001), except for c702 BCG vs. c702 BCP (*p*-value 0.818), indicating that calculated FT would not be influenced by albumin dye method used on the c702. Calculated FT was compared to FT by ED-LC-MS/MS in men and was found to be significant only for the ARCHITECT BCG method (*p*-value 0.034). **Conclusions:** Comparisons of the albumin methods show that they are statistically different. However, when albumin is used for determination of FT, most do not show a significant impact, as indicated by the comparison of calculated and directly measured FT in men. Due to the potential significant differences that could occur, careful evaluation should be considered when selecting and switching albumin dye methods; the clinical use of serum albumin testing and the patient populations served by the laboratory should be considered.

**A-204**

Assessment of full thyroid profile in pediatric primary care focusing on the use of serum thyrotrpin and free thyroxine only. Less is more?

V. Zaidman, J. Lazzati, M. Maceiras, V. Herzovich, A. Belgorosky, E. A. Chaler, Hospital de Pediatrica Garrahan, Buenos Aires, Argentina

**Background:** It has been widely accepted that the major changes in thyroid function in adult subjects may initially be studied with the assessment of serum thyrotropin (TSH) and free thyroxine (FT4) only; however, there is little evidence whether the same criteria can be used in the pediatric population. Objective: The aim of this study was to describe the distribution of results obtained and subsequent medical management in a pediatric population assessing serum full thyroid profile focusing on serum TSH and FT4 only.

**Methods:** We retrospectively analyzed consecutive patients in whom thyroid function was studied with a full thyroid test seen between November 2014 and September 2015. Samples were tested on the day at the same laboratory. We excluded those subjects who were not seen by a physician after the studies, those who were treated with levothyroxine, methimazole, or antiepileptic drugs, and seriously ill patients. The remaining 5739 subjects were categorized according to age from one week to 12 years. TSH, T3, and FT4 were measured with Architect i4000 (Abbott) and total T4 IMMULITE 2000 (Siemens).

**Results:** Subjects with serum TSH and FT4 within the reference interval (RI) according to age were selected (n = 4008, 69.83% of total). From this group, children who had T3 and/or T4 outside the RI were selected (n = 391, 9.75%). For both analytes, 9.75% of healthy subjects is statistically expected to be found outside the RI (0.05%). In the clinical chart (CC) of 381 patients (97.4%) no comment was made on thyroid function or the thyroid profile was reported to be normal, in spite of T3 and/or T4 being outside the RI; maybe because in all cases T3 and/or T4 were within the reference change value (RCV) on the limits of the RI. In 10 patients (2.6%) a comment was made in the CC; five patients were sent home without further interventions, in one patient with high T3 and T4 high serum levels of Thyroxine-Binding Globulin were found, and four were finally treated with levothyroxine. All of them had T4 and FT4 at the 10th percentile of the RI and two additionally had TSH above the 90th percentile of the RI.

**Conclusion:** Of the study population, 69.83% presented with normal TSH and FT4 levels. Of these patients, 9.75% had T3 and/or T4 outside the RI, which is statistically acceptable for a normal population. As to the 10 patients that had some comment in their CC regarding test results, only four were put on treatment, and all had FT4 below the 16th percentile and two had TSH above the 90th percentile as well. Screening of serum TSH and FT4 only may be useful in children tested to rule out thyroid disease. It is recommended to assess FT4 and TSH results with a stricter RI with a range between the 10th and 90th percentile considered acceptable. A prospective study in a pediatric population would be necessary to clinically confirm these results as well as the subsequent medical management.
**A-205**

**Plasma calcitonin: maintaining assay harmonisation following method transfer**

X. Hong, J. Ellis. *Canterbury Health Laboratories, Christchurch, New Zealand*

**Background:** Plasma calcitonin is known to be an unstable analyte and vulnerable to repeated freeze-thaw cycles, incurring risk of sample rejection through thawing in transit when sent to another centre. We therefore undertook a method comparison between the Siemens Immulite method employed at the referral centre and the Roche platform, with a view to enable processing within our own laboratory. Roche Elecsys Cobas and Siemens Immulite are both two site immunometric assays that measure the mature 32 amino acid monomer of calcitonin. Both methods are calibrated to WHO 89/620 and with similar sample requirements. **Objective:** To compare calcitonin measurement by the Roche and Siemens assays and examine the clinical concordance of results. To examine the effect of freeze-thaw cycles on calcitonin measurement.

**Methods:** Clinical specimens (n=47) were exchanged between the two laboratories (June 2013 to December 2014). All samples were stored and transported frozen. Three patient samples with different calcitonin levels were run 10 times (9 times for one sample as it was insufficient for the tenth run) on the same day to assess within run imprecision. Another 3 samples with different levels of plasma calcitonin were analysed over 15 days to test for between run imprecision. Three different samples with high calcitonin levels were diluted serially to test for assay linearity. To justify the validity of including twice thawed samples in the method comparison, 9 samples that had not been thawed previously were subjected to repeated freeze-thaw cycles (n=4) and calcitonin measured. **Results:** 27 out of 47 samples had detectable calcitonin, and the remaining samples were below detection limit (<0.5 ng/L) by the Roche Calcitonin method. 5 out of 47 samples were paired (2 aliquots from the original patient samples were made before freezing) and the remaining 42 samples had undergone two freeze-thaw cycles before measurement on the Roche analyser. The Roche calcitonin assay agreed well with the Siemens Immulite method statistically [Slope=1.03 (0.98 to 1.09), Intercept=0.05 (+0.65 to 0.75) by Weighted Deming Regression]. There was good clinical concordance between results from the two laboratories (n=47). Within run imprecisions (CVs) were 3.4%, 9.2% and 10.4% for the 3 samples, with between run imprecisions (CVs) being 9.9%, 10.3% and 12.9%. The Roche assay showed satisfactory dilution linearity, with mean +/- SD of diluted calcitonin = 94% +/- 10.2% using the Roche assay. **Conclusion:** It was valid to have included twice thawed samples in the calcitonin method comparison. The Roche calcitonin assay agreed well with the Siemens Immulite method both clinically and statistically. Harmonisation can therefore be maintained by transferring from the Siemens Immulite to the Roche Elecsys/Cobas calcitonin assay.

**A-206**

**Is one-minute microcentrifugation of samples ensuring rapid and reliable results for intraoperative PTH measurement in a routine lab?**

A. Bothy, R. Debois. *CHU UCL Namur, Namur, Belgium*

**Background** Intraoperative parathyromone (ioPTH) monitoring is now a common practice and a guide for surgical decisions during parathyreodectomy. With a half-life of only few minutes, PTH levels directly reflect in vivo activity of parathyroid tissue and provide assurance that all hyperfunctioning tissues have been removed. According to the Miami criteria, PTH levels should be assessed just before excision as a baseline value and success of surgery is defined as a fall of ≥ 50% from the baseline at 10 minutes post-excision. If the criterion is not met additional measurement at 20 minutes or extended neck exploration is necessary. The goal of this study is to improve turnaround time of ioPTH laboratory testing by shortening preanalytical phase.

**Methods** Whole blood specimens from 29 parathyroid surgeries were collected on EDTA tube at different time intervals. For each intervention, a pre-skin incision and/or pre-gland excision sample was obtained and represented baseline value. The number of intraoperative specimen varied from 2 to 10, with a total of 163 analyzed samples. Each sample underwent a one-minute microcentrifugation at 13000 rpm before performing PTH STAT assay on the Roche Cobas-6000 analyzer. At the same time all specimens were tested after a 10 minutes centrifugation step as initially done in our laboratory.

**Results** Statistical analysis showed an extremely significant and positive correlation coefficient (r=0.997, p< 0.0001) between the two methods. The mean difference observed on Bland and Altman plot was 9.9 ng/L (95% CI: −16.6 to 36.4). The Passing and Bablok regression analysis provided a slope of 0.91 (95% confident interval: 0.90 to 0.92) and an intercept of 0.51 (95% confident interval: −0.18 to 1.24), meaning that no systematical but a slightly proportional difference was observed.

**Conclusion** This study shows that reliable results of ioPTH are obtained with minimal pre-analytical phase. Implementation of this new procedure in our laboratory along with close collaboration with surgical teams will have direct benefits on turn-around time and patient care.

**A-207**

**Development of a High-Sensitivity Prototype Assay for Thyroid Stimulating Hormone (TSH) on the VITROS® 3600 and ECi/ECiQ Immunodiagnostic and 5600 Integrated Systems**

P. Hosimer, A. Gardner, A. Stroud, E. Oliver, M. Kantar. *Ortho Clinical Diagnostics, Rochester, NY*

**Background:** A new prototype assay for thyroid stimulating hormone (TSH) is under development for the VITROS 5600, 3600 and ECi/ECiQ Systems. The prototype assay targets a faster time-to-result with reduced sample volume, expanded measuring range and improved sensitivity.

**Methods:** All testing was conducted on the VITROS 3600 and ECiQ Immunodiagnostic Systems. LoB/LoD/LoQ and precision were evaluated using modified CLSI protocols. Five precision pools were evaluated, with TSH values ranging from 0.105 to 71 µIU/mL. Precision testing occurred in ten runs on two instruments over a period of five days, with two replicates per run (n=40). Accuracy of the prototype assay was evaluated in a method comparison study by testing 120 serum samples ranging from 0.002 to 189 µIU/mL TSH. Samples were tested on the prototype assay and the current VITROS Immunodiagnostic Products TSH assay with an extended calibration range. Passing-Bablok regression was used to analyze the results.

**Results:** The prototype assay was determined to have LoB = 0.0005 µIU/mL, LoD = 0.0025 µIU/mL and LoQ (20% CV) = 0.0031 µIU/mL with a time-to-result of 24 min. Testing of the five precision pools (n=40) produced total imprecision ranging from 5.0 to 6.4 %CV. Analysis of the method comparison study (n=120) generated a slope of 1.027 and y-intercept of 0.0019.

**Conclusion:** The results demonstrate that the prototype TSH assay is accurate, precise and sensitive, while delivering assay results 40% faster than the current TSH assay.
Identification hemoglobin variants during glycosylated hemoglobin measurement

S. I. Villanueva-Herráiz1, M. J. Vélez-González2, E. Lepe-Balsalobre1, E. M. García-Agudo3, M. D. Vitoria-Peñas1, J. M. Guerrero-Montávez2, A. Moro-Ortiz2, Virgin de Valme University Hospital, Seville, Spain, 2Virgen del Rocío University Hospital, Seville, Spain

Background: Glycated hemoglobin, measured as hemoglobin A1c (HbA1c), is the most reliable marker for monitoring long term glucose control in patients with diabetes mellitus (DM). Hemoglobinopathies do not usually have a clinical impact buy they can interfere with the analytical determination of some parameters such as the HbA1c. Many hemoglobin variants (HbV) are detected incidentally during the measurement of HbA1c.

The aims of this study were to describe HbV detected during HbA1c measurement since the introduction of the Hb-Advisor™ application (instrument Manager v.8.09, Data Innovations LLC) and to check if affect the accuracy of the HbA1c measurement.

Methods: Descriptive study, from 2012 to April 2014. HbA1c quantification was performed by HPLC assay on Bio-Rad VARIANT II™ TURBO HbA1c Kit-2.0 (Hercules, CA, USA) system with the 1.5 min. Screening abnormally chromatogram was done with the computer application Bio-Rad Hb-Advisor. Predefined rules warning were: anomalous peaks; P3 or P4< 7%; HbA1c> 2.6%; HbA2< 3.5%; HbA1c< 4% or >11.5%; Labile HbA1c< 4% and fetal hemoglobin (HbF)>10%. In those samples with anomalous chromatograms, HbA1c was measured again using a method based on a different analytical principle: boronate affinity chromatography (Afinion™ AS100; Axis-Shield, Oslo, Norway), HPLC with Bio Rad VARIANT II™ thalassemia Short Program and immunoagglutination method DCA 2000 (Bayer, Vienna, Austria)

We reviewed laboratory parameters: routine biochemistry and hemogram parameters. Since the abnormal chromatography indicated a suspected HbV, blood samples were sent to a thalassemia and hemoglobinopathy reference laboratory for: Hemoglobin was assessed by capillary zone electrophoresis on Sebia Capillarys Flex system using the Capillary Hemoglobin kit (Sebia, Norcross, GA, USA). Globin chains were identified by reverse-phase HPLC using a Vydar large-pore C4 column (The Separations Group, Hesperia, CA, USA). Functional studies were carried out with a Hemox-Analyzer TCS Medical Products (Huntingdon Valley, PA, USA). Hemoglobin stability test with isopropanol. For molecular characterization of the gene, genomic DNA from isolated leukocytes was extracted using an automatic extractor (BioRobot EZ1; Qiagen).

Results: We analyzed 121424 samples, median 4562.6 [3162-5765] samples/month. We detected 84 HbV, 62 were identified. Median age 51.5 (10-86) years, 51.2% women. 22.7% non-Caucasian. Hemoglobinopathies identified (all heterozygous):

** Conclusion: The use of applications such as Hb-Advisor™ allows us to discriminate extreme values of HbA1c or anomalous chromatograms. In such cases, a visual inspection of the HPLC chromatogram may give valuable information regarding HbV, allowing the detection of inaccurate results. When the laboratories suspect inaccurate results that may lead to over- or under-treatment of diabetic patients, choosing an alternative non-Hb-based methods for assessing long-term glycemic control (such as fructosamine assay) may be useful. It is important to know those HbV that interfere with HbA1c measurement method used.

Characterization of 1.5-Anhydroglucitol levels in Brazilian adult subjects without diabetes

E. S. Gomes1, N. M. Sumita1, J. G. Vieira1, M. Nery1, M. G. T. Bezerra1, 1Clinical Endocrinology Division, Diabetes Unit of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP) and Fleury Medicina e Saúde, São Paulo, Brazil, 2Central Laboratory Division (LIM-03) of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP) and Fleury Medicina e Saúde, São Paulo, Brazil, 3Clinical Endocrinology Division, Diabetes Unit of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP) and Fleury Medicina e Saúde, São Paulo, Brazil

Background: 1.5-Anhydroglucitol or 1.5-AG is a glucose-like monosaccharide contained in food and it is a validated marker of short-term glycemic control. During periods of hyperglycemia, glucose blocks reabsorption of 1.5-AG in the renal tubules. Thus, low blood levels of 1.5-AG are associated with hyperglycemia. 1.5-AG may predict more rapid changes in glycemia than glycated hemoglobin -A1C or fructosamine. Since 1.5-AG levels can be influenced by racial or ethnic groups, reference values should be specific for each population. Aim: to establish the 1.5-AG levels in Brazilian adult patients without diabetes.

Methods: a group of population without clinical and laboratory evidence of diabetes was evaluated. The study was submitted and approved by our Internal Review Board. The blood samples of 986 subjects, 506 women and 460 men, aging 40 ± 10 years, were analyzed. The blood was collected by venipuncture after 12 hours fast. The samples were collected in serum gel evacuated tubes for 1.5AG and glucose tests and EDTA tubes for A1C -Vaccutek, Greiner Bio One International GmbH, Germany. Serum 1.5AG was measured using the GlycoMark assay - GlycoMark, Inc., Japan and glucose using enzymatic hexokinase method by Roche. The analysis were carried out on cobas 8000 modular analyzer series, C702 module -Roche Diagnostics GmbH, Germany. The HPLC method certified by NGSP was used to measure the A1C level on Tosoh automated glycohemoglobin analyzer HLC-723G8 -Tosoh Corporation, Japan.

Results: the mean results in the whole population were 1.5 AG: 15.89±6.47 µg/mL, glucose: 89±6 mg/dL and A1C: 5.2± 0.3%. Sex-specific mean results were 1.5 AG: 16.71±6.59 µg/mL, glucose: 90±5 mg/dL and A1C: 5.3± 0.3% for men and 1.5 AG: 15.14±6.26 µg/mL, glucose: 88±6 mg/dL and A1C: 5.2±0.3% for women.

Conclusion: in Brazilian patients without diabetes the reference intervals, 2.5th 97.5th percentiles were 11.68-29.18 µg/mL for males and 10.54-28.94 µg/mL for females. Since we were able to establish reference values in a Brazilian population, the use of 1.5AG assay would be suitable an alternative marker for glycose monitoring in the short-term glycemic control allowing rapid practitioner intervention in uncontrolled diabetic subjects.

Free T3 and Total T3 Assays with Six Point Calibrators on the ARCHITECT Analyzer (In Development).


BACKGROUND: In a clinical setting, thyroid function is often evaluated through testing for thyroid stimulating hormone (TSH) and other thyroid hormones such as triiodothyronine (T3) and thyroxine (T4). In the particular situation where the TSH result does not agree with the T4 result and thyroid disease is suspected, additional testing for T3 (FT3 and TT3) may be needed to confirm diagnosis. T3 is bound to endogenous proteins such as thyroxine binding globulin, pre-albumin and albumin; and as a result, a small portion of T3 is free in the bloodstream. This fraction represents the active form of the hormones. The ARCHITECT Free T3 and Total T3 assays were developed to aid in the diagnosis and monitoring of hyperthyroidism. METHODS: The ARCHITECT Free T3 and Total T3 assays are two-step immunoassays to determine the presence of free (unbound) T3 and Total T3, respectively, in human serum and plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology. In the first step, sample and anti-T3 coated paramagnetic microparticles are combined. Free T3 (unbound) or Total T3 present in the sample binds to the antiT3 coated microparticles. After washing, T3 acridinium labeled conjugate is added in the second step. Pre-Trigger and Trigger Solutions are then added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). An inverse relationship exists between the amount of Free T3 or T3 in the sample
Monitoring of the standardization and harmonization status of FT4 and TSH assays by use of patient medians.

1Department of Pharmaceutical Analysis, Ghent University, Ghent, Belgium, 2RefAU, Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium. 3Thienpont & Stöckl Wissenschaftliches Consulting GbR, Rennertshofen, Germany

Background: For the diagnosis of thyroid dysfunction clinicians rely in first instance on TSH and FT4 testing. However, the lack of comparability between assays is an issue for patient safety. Therefore, the IFCC Committee for Standardization of Thyroid Function Tests (C-STFT) is involved in standardization/harmonization efforts. Once assay recalibration to generate comparable results will be accomplished, the C-STFT will be challenged to monitor sustainability of this status. We believe this will be possible by applying our recently developed Percentiler and Flagger. They are intended to serve as quality indicator for stability of performance (Percentiler) and flagging rate (Flagger). Although the recalibration of FT4 and TSH assays is not yet completed, we considered it worth to start applying the tools in the pre-implementation phase, because it would allow us to timely recruit participants and build experience.

Methods: For the Percentiler, laboratories calculate and electronically send us instrument-specific daily medians from outpatient results. For the Flagger, daily flagging rates (± hypo and hyper) are reported. We developed software and user interfaces to plot the course of the instrument-specific moving medians (Percentiler) and flagging rates (Flagger) in comparison to instrument-based peer group medians. Laboratories have access via a lab-specific password. They see their own plots and data, including their long term median(s) in comparison to the peer group. Laboratories can visually infer whether the performance of their instruments and of their peer is stable, i.e., when the moving medians remain between desirable bias limits proposed by us. For the Percentiler, these are guided by biological variation and state-of-the-art performance, i.e., ±3.3% for FT4 and ±7.7% for TSH, respectively. For the Flagger, the limits are preliminary set at ±30% of the long-term flagging rate.

Results: In the Percentiler, we currently have 77 participants with 147 instruments, from which we distinguish 4 peer groups (n = 15); in the Flagger, only 29 laboratories with 38 instruments participate. From our short time experience we see already that for FT4 and TSH the moving medians are more variable than for clinical chemistry analytes; in spite of this, most laboratories have satisfactory stability of performance. Nevertheless, we sometimes observe significant shifts due to lot changes, differences between instruments, drifts or saw-tooth patterns due to reagent instability. Since we group laboratories according to instrument peer, we can also monitor/compare the stability of instruments. We also see the interplaying effect between the Percentiler and Flagger, i.e., an increase of the median values in the Percentiler results in a decrease of the %-hypo and increase of the %-hyper in the Flagger, and vice versa.

Conclusion: By starting to use the Percentiler and Flagger applications for the C-STFT, we showed their utility for monitoring the stability of FT4 and TSH performance in laboratories and instruments and the effect of instability on the flagging rate. This gives evidence that the tools will also be useful to document the sustainability of the post-standardization/harmonization status. Nonetheless, we need to recruit more participants, so that all instruments participating in the C-STFT activities are represented.

A-212

Establishing reference intervals for hCG in postmenopausal women

K. Patel, A. Qavi, K. Hock, A. Gronowski. Washington University, Saint Louis, MO

Background: Human chorionic gonadotropin (hCG) screening tests are performed in nearly all female patients prior to any medical intervention regardless of age. Plasma hCG concentrations have been shown to increase with age due to pituitary secretion, resulting in a positive hCG in the absence of pregnancy. We previously reported that an hCG cutoff of 14.0 IU/L be used for women >55 years of age. However, it remains unknown whether concentrations greater than 14.0 IU/L can be expected in women with advanced age. Additionally, the relationship between FSH and hCG has not been examined in postmenopausal women. Our objectives were to establish serum hCG reference intervals and correlate FSH and hCG concentrations in non-pregnant postmenopausal females age >55 years.

METHODS: A total of 796 residual plasma samples from women >55 years were collected with 303, 269, and 224 samples belonging to the age groups 55-69, 70-84, and >85 years, respectively. FSH and hCG were measured using the Abbott Architect.

A-213

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A-211

Elecsys® AMH immunoassay: Evaluation of the novel assay’s precision under routine conditions

A. Algeciras-Schminich1, A. D. Pierce2, M. A. Lessig2, D. Pardue2, R. E. Ostlund2, 1Clinical Immunoassay Laboratory, Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, 2Nationwide Laboratories Services, Fort Lauderdale, FL, 3Roche Diagnostics Operations, Indianapolis, IN, 4Division of Endocrinology, Metabolism and Lipid Research, Washington University, St Louis, MO

Background: Measurement of ovarian reserve levels plays a key role in predicting treatment response to controlled ovarian stimulation (COS) in assisted reproductive therapy. Anti-Müllerian Hormone (AMH), released from ovarian granulosa cells leading to serum levels proportional to the number of developing follicles in the ovaries, is a promising marker for assessing the ovarian reserve and optimizing in vitro fertilization treatments. Currently available manual AMH assays exhibit limitations with respect to run time and reliability of results. The Elecsys® AMH assay is a fully automated sandwich electrochemiluminescence immunoassay for the in vitro quantitative determination of AMH in human serum and lithium heparin plasma. The aim of this study was to evaluate the technical performance of this immunoassay in terms of precision under routine conditions.

Methods: Three laboratories in the United States evaluated the reproducibility performance of the assay according to CLSI EP-15-A2 guidelines using a cobas e 411 analyzer. Precision experiments were performed with Human Serum Pools (HSP) generated using human serum from postmenopausal women (BIOMEX GmbH), known to contain very low levels of AMH. Tested AMH concentrations, covering major parts of the measuring range (0.2-20 ng/ml [1.4-143 pmol/L]) were obtained by adding fetal bovine serum with high levels of AMH to these pools. Subsequently, the pools were aliquoted and stored frozen until measurement at the respective sites. In total, the analyzed imprecision pool consisted of five HSPs (HSP 01-05) and two PreciControl AMH samples (PC01=1.0 ng/mL [7.14 pmol/L] and PC02=5.0 ng/mL [35.7 pmol/L]). The sample pools were tested in replicates of three in one run per day.

Results: For the reproducibility experiments, the CVs (95% CI) were shown to be <5.5% across all three sites (HSP01 3.99% [2.68-7.7], HSP02 4.05% [2.79-7.42], HSP03 3.45% [2.33-6.66], HSP04 4.62% [3.01-9.80], HSP05 4.38% [2.94-9.80], PC01 5.24% [3.46-10.6] and PC02 4.70% [3.18-8.91]. The CVs for repeatability were all <2% (HSP01 1.58% [3.14-1.92], HSP02 1.74% [1.48-2.12], HSP03 1.41% [1.19-1.71], HSP04 1.69% [1.44-2.06], HSP05 1.69% [1.43-2.06], PC01 1.42% [1.21-1.73] and PC02 1.89% [1.60-3.30]).

Conclusion: The fully automated Elecsys AMH immunoassay run on the cobas e 411 under routine conditions demonstrated very good precision performance in the concentration range of 0.2-20 ng/mL (1.4-143 pmol/L). Consequently, the availability of this immunoassay will represent a fast and reliable alternative to conventionally used manual methods for AMH testing.

Disclaimer: This product is not cleared or approved for use in the USA.
Patients with FSH <8.0 IU/L were assumed to be on hormone replacement therapy and were excluded. All positive ICG samples were analyzed for heterophile antibody interference and 3 were excluded. Patient chart review was performed for each positive hCG sample. 4 were excluded due to malignancy.

RESULTS: 10% (67/676) of women age >55 years had plasma hCG >5 IU/L. There were 22, 21, and 24 patients with hCG >5 IU/L in the age groups 55-69, 70-84, and >85 years, respectively. The hCG concentrations observed in each age group were as follows: 55-69 years maximum =12.4 IU/L and 97th percentile = 11.7 IU/L; 70-84 years maximum = 18.09 IU/L, 97th percentile = 9.32 IU/L, and >85 years maximum = 11.1 IU/L and 97th percentile = 10.9 IU/L. We found no correlation between hCG and FSH concentrations. Additionally, hCG concentrations did not continue to increase with age over 55 years.

CONCLUSIONS: In women>55 years of age, plasma FSH concentrations do not predict hCG concentrations and age-specific reference intervals are not needed beyond 55 years. This study confirms that our previously recommended cutoff of 14 IU/L should be used for women>55 years of age.

### A-215

**Use of liquid chromatography/tandem mass spectrometry to assess diurnal effects of steroids and neurosteroids**

K. J. Welsh1, B. R. Stolze2, L. S. Masika1, K. Nayak2, M. Sigdel2, J. Jonklaas3, S. J. Soldin1. 1National Institutes of Health, Bethesda, MD, 2Georgetown University, Washington, DC

**Background:** Alterations of the hypothalamic-pituitary-adrenal system are reported in a number of conditions. Diurnal variation has been demonstrated for cortisol and a number of steroids using liquid chromatography-tandem mass spectrometry (LC-MSMS) with significantly higher circulating levels in the morning, indicating the need to develop time-specific reference intervals. Neurosteroids are a class of steroids with central nervous system modulation activity. The neurosteroids allopregnanolone, dehydroepiandrosterone (DHEA), tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone are key mediators of the stress response and are linked to several neurologic, endocrine, and psychiatric disorders; however, diurnal variation of these neurosteroids and reference intervals have not been previously evaluated. Our aim is to develop reference intervals for LC-MSMS measurement of a steroid profile and neurosteroids, and assess if diurnal variation could also be observed in neurosteroids.

**Methods:** Early morning serum samples were collected between 6:00 am to 8:00 am, and evening serum samples between 6:00 pm to 8:00 pm from 24 healthy volunteers. Study volunteers were generally healthy with no current or past major illnesses. LC-MSMS was used to measure a steroid profile that includes androstenedione, cortisol, corticosterone, cortisol, dehydroepiandrosterone (DHEA), tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone. The neurosteroids allopregnanolone, DHEA, tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone were also measured. The paired sample Wilcoxon test was used to compare differences between morning and evening values.

**Results:** The median values and 2.5th - 97.5th percentiles for morning and evening steroid levels are shown in Table 1. Statistically significant differences were observed between morning and evening values for all steroids and the neurosteroid DHEA. The neurosteroids allopregnanolone, tetrahydro-11-deoxycortisol, and tetrahydro-11-deoxycorticosterone were undetectable in most of the normal volunteers.

**Conclusions:** Time-specific reference intervals are essential to evaluate the clinical relevance of steroid profiles and the neurosteroid DHEA.

<table>
<thead>
<tr>
<th></th>
<th>Morning (2.5th percentile)</th>
<th>Evening (97.5th percentile)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione</td>
<td>66.5 (13.3 - 188.0)</td>
<td>46.5 (28.2 - 186.1)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Cortisol</td>
<td>12.0 (0.5 - 20.8)</td>
<td>3.95 (1.8 - 11.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>1.41 (0.46 - 5.0)</td>
<td>0.45 (0.1 - 1.6)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cortinine</td>
<td>20.9 (11.9 - 29.3)</td>
<td>8.1 (3.9 - 23.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DHEA</td>
<td>239.0 (61.5 - 693.0)</td>
<td>191.0 (43.0 - 438.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>11-deoxycortisol</td>
<td>59.0 (0.6 - 112.5)</td>
<td>11.6 (0.0 - 59.4)</td>
<td>0.0012</td>
</tr>
<tr>
<td>17a-hydroxyprogesterone</td>
<td>20.8 (1.1 - 158.2)</td>
<td>11.5 (0.0 - 154.9)</td>
<td>0.0006</td>
</tr>
</tbody>
</table>

### A-216

**Comparison of HbA1c values from the Alere Afinion and Tosoh G8 HbA1c Analyzers Before and After Tosoh Assay Recalibration**


**Background:** Analysis of hemoglobin Alc (HbA1c) is a cornerstone in the management of patients with diabetes. Although Point-of-Care (POC) HbA1c devices are not recommended for use in the diagnosis of diabetes, they are used frequently in physician offices and clinics to monitor the glycemic control of patients. An added benefit of POC analyzers is the rapid analysis time, which allows clinicians to give a result to the patient during the office visit and counsel the patient based on that result. Our institution uses two methodologies for measuring HbA1c: a HPLC method (Tosoh G8) in the laboratory and a POC boronate affinity method (Alere Afinion) at satellite locations. It is not uncommon for diabetic patients in our hospital system to be seen at different clinic locations and have HbA1c testing done by both methods. All methods for measuring HbA1c undergo an accuracy based evaluation with comparison to National Glycohemoglobin Standardization Program (NGSP) reference methods. Historically, when the results of the Tosoh peer group had been compared to the reference method in College of American Pathologists (CAP) surveys, the results had been consistently higher than the reference method. In response to customer concerns, in 2015 the manufacturer recalibrated the Tosoh assay to bring HbA1c values closer to the reference method. In contrast, its closer to the reference method. In contrast, its closer to the reference method. The Tosoh assay has been shown to have a negative bias compared to reference methods when measuring HbA1c values greater than 8%. As many of our patients have HbA1c values above 8% and could have measurements performed by both instruments, we wanted to compare values from both methods before and after the Tosoh recalibration.

**Methods:** Analysis of data from comparison studies of HbA1c results assayed by the Afinion and the Tosoh before and after recalibration in 2015. Pre-recalibration data included initial validation and method comparison studies between the Tosoh G8 and the Alere Afinion HbA1c analyzers. Post-recalibration data was gathered from method comparison studies performed on new lots of Afinion cartridges.

**Results:** Initial method comparison studies showed that results from the Afinion had a negative bias when compared to the Tosoh (y=0.9531x-0.2164). This was especially pronounced at values above 8%, where the majority of specimens from the Tosoh were higher than the Afinion. After a preliminary evaluation of the data comparing the Afinion to the Tosoh after manufacturer recalibration of the assay, this bias has decreased, but is still present (y=1.0197x-0.3396). Further analysis will be done to focus on HbA1c values greater than 8%. **Conclusions:** After recalibration of the Tosoh assay, the agreement between the two assays, particularly at values above 8%, is better.

### A-218

**Derivation of the biologic variation of data-mined hCG**

J. Mei1, G. S. Cembrowski1, T. Higgins2, K. Moses1, A. Fuezyer1. 1Alberta Health Services, Edmonton, AB, Canada, 2DynaLife, Edmonton, AB, Canada

**Introduction:** Most estimates of biologic variation (s_b) are based on acquiring and storing specimens from reference subjects, followed by analysis within a tightly controlled analytic run. The s_b of certain analytes, such as HCG, cannot be easily derived in this manner as hCG is produced in select subjects over a relatively short period. We propose that data-mined intra-patient hCG results can be statistically analyzed to provide s_b.

**Methods:** A data repository provided all outpatient and inpatient HCG measured over 5 years at a large referral laboratory in Edmonton and at 5 Edmonton hospitals. The HCG measurements were made with Roche (4th WHO IS) and Beckman (3rd WHO IS) hCG analyzers for the referral and hospital patients, respectively. Patient hCG results were analyzed if at least two inpatient results were obtained within 84 hours. Three different HCG ranges were studied: under: 1,000 IU/L, 1,000 to 10,000 IU/L and 10,000 to 100,000 IU/L. We tabulated the pairs of intra-patient hCG that were separated by 0-6, 6-12, 12-18, ... 72-78 and 78-84 hours. We calculated the standard deviations of duplicates (SDD) of the paired data. The SDD were regressed against the midpoints of the time intervals. While the y intercept represents the sum of s_b and short term analytic variation (s_y), we assumed that short term imprecision was negligible compared to s_b.

**Results:** The Table summarizes the results; CV_y is derived from the ratio of s_b to the median HCG. While the CV_y are plausible for the two lower hCG concentrations, the Roche and Beckman s_b are highly divergent for the high concentrations. These differences may be attributable to the Roche system’s wider linear range.
Evaluation of a new automated method for glycosylated hemoglobin on the Abbott Architect C8000

M. Lee1, L. Lam1, S. K. Phua1, T. C. Aw1. ‘Ng Teng Fong General Hospital, Singapore, Singapore, ‘Changi General Hospital, Singapore, Singapore

Background: We verified the analytical performance of an enzymatic HbA1c assay on the Abbott Architect C8000 Chemistry System (Abbott) and compared it to a contemporary immuno-turbidimetric assay on the Cobas 502 (Roche).

Methods: In the Abbott HbA1c assay glycosylated N-terminal dipeptide (fructosyl-VH) of the hemoglobin beta chain is cleaved by the addition of protease. Fructosyl-VH then reacts with peroxidase and fructosyl peptide oxidase. HbA1c concentration is measured by determining the resultant hydrogen peroxide. The Abbott HbA1c was verified for imprecision, linearity and accuracy. Imprecision was carried out by measuring 2 levels of QC material (BioRad) in triplicate over five days in accordance to CLSI EP2-A2 guidelines. Linearity and accuracy were assessed by analysing 5 HbA1c specimens ranging between 4.0-15.0% in triplicate. All data are evaluated using EP Evaluator Software.

Consecutive patient samples (n=168) with a range of HbA1c values - below 5.6% (non-diabetes-50), 5.7-6.4% (prediabetes-50), above 6.5% (diabetes-68) - were analysed on both the Abbott and Roche platforms and compared. Statistical analyses were performed using MedCalc v16.2.0 software (Ostend, Belgium).

Results: Assay imprecision (CV) for control materials at levels of 5.4% and 10.0% HbA1c were 1.1% and 1.5% respectively. The Abbott assay is linear across the manufacturer’s claimed measuring range of 4.0-15.0% (slope=1.020, intercept=-0.11) and sufficiently accurate (maximum deviation for a mean recovery from 100% was 3.1%).

For the assay comparisons samples from 79 women and 89 men were used (age: range 17-99, mean 57.8, SD 19.0 years respectively). Passing-Bablok Regression: Abbott = 1.025641 Roche = 0.235897; Cusum test for linearity: No significant deviation from linearity (P=0.06); r = 0.99 (95% CI 0.9865 to 0.9926). Bland-Altman plots revealed a mean absolute difference (Abbott-Roche) in HbA1c values of 0.01% (range -0.75 to +0.77%). For HbA1c samples below 6.5% (n=100), the mean difference was 0.08% (range: -0.33 to +0.36) and for HbA1c samples over 6.5% (n=68), the mean difference was 0.16% (range: -0.95 to +1.26).

Conclusion: The imprecision of the Abbott HbA1c assay is well within the NGSP targets of <2.0% CV for samples targeted at 6.5% HbA1c and ≤5.3% CV for samples over 7.0% HbA1c. There was close agreement between the Abbott and Roche A1c assays. The new Abbott HbA1c assay offers clinical laboratories an additional choice for HbA1c measurement.

Clinical Utility of Aldosterone, Renin Mass and the Aldosterone/Renin Ratio for the work up of suspected Primary Aldosteronism

J. R. Wiencek1, S. Stafford1, A. Woodworth1. ‘Vanderbilt University School of Medicine, Nashville, TN, ‘Vanderbilt University, Diagnostic Laboratories, Nashville, TN

Background: Primary aldosteronism (PA) is a group of disorders characterized by inappropriate aldosterone production. PA is caused by adrenal adenoma or hyperplasia. The Endocrine Society recommends a multi-tiered approach for the diagnosis of PA in high risk patients (i.e. drug resistant hypertension, hypokalemia and/or hypertension with adrenal incidentaloma) by: (1) Screening, via calculation of the aldosterone/renin ratio (ARR), and (2) Abnormal ARRs should be confirmed with provocative testing and adrenal vein sampling. The accepted method for renin quantification is by plasma renin activity (PRA). New assays are available that quantitate renin mass by immunoassay (Direct Renin Concentration or DRC).

Objective: To compare the clinical utilities of renin measured by the activity or mass assay to predict primary aldosteronism.

Methods: A retrospective cohort study was performed utilizing residual plasma-EDTA specimens of 200 hypertensive adults (134 female and 66 male) being worked up for PA. Leftover specimens sent to the Vanderbilt University Medical Center Esoteric Chemistry laboratory with orders of PRA and plasma aldosterone concentrations (PAC) were utilized. Specimens were included from patients ≥18 years with hypertension and an order for PRA and/or PAC. Plasma-EDTA specimens were frozen until analysis. Review of the electronic medical records was conducted to determine patient history, diagnosis, and outcomes. The gold standard for PA diagnosis was based on ES guidelines of abnormal ARR (PRA cutoff >30 or DRC >5.7), PAC (>15ng/dL) and provocative conﬁrmatory testing. PAC and DRC were measured by immunoassay on theDiaSorin Liaison. PRA was measured with DiaSorin’s radioimmunoassay kit. Stability studies were performed at different storage conditions for PAC and DRC. The clinical utility of the ARR calculated with either PRA or DRC (ARR-PRA or ARR-DRC) to predict PA was assessed by Receiver Operating Characteristic (ROC) analysis, using GraphPad software.

Results: PAC and DRC were stable for at least two months at -80°C. PRA and DRC results correlated well, r = 0.95. Of the 200 patients, 8 had likely diagnosis of PA (2 confirmed by gold standard, 3 with an elevated ARR, aldosterone and adrenal nodule, and 3 with physician documented diagnosis). ROC analysis demonstrated that ARR-PRA and ARR-DRC showed similar ability to predict PA; areas under the ROC curves were 0.98 and 0.95 respectively. At the ES recommended cutoffs, sensitivity, specificity and positive-likelihood ratios (LR+) were 100% (95% CI), 78% (72 - 84), and 4.6 respectively for ARR-PRA and 62% (59 - 65 [95% CI], 96% (92 - 98), and 15.2 respectively for ARR-DRC. Specificity and LR-+ improved with the use of both the elevated ARR and aldosterone to 95.5% (92 - 98) and 19.4 for ARR-PRA and 97% (93 - 99) and 20.3 respectively for ARR-DRC. False positive ARRs were seen with kidney disease, no follow up testing, and/or interfering medications.

Conclusion: The combination of elevated aldosteronism and ARR (calculated with PRA or DRC) showed superior clinical utility to predict PA in high-risk patients. Because of false positives, interfering PA must be confirmed with provocative testing. Performance characteristics were similar for the DiaSorin DRC and PRA assays. Furthermore, DRC testing is rapid, stable overnight and eliminates the need for radioisotopes.

Age specific TSH reference ranges generated by electronic medical record database mining: data from over 33,000 healthy patients

J. C. Dregs, M. S. Petrie, K. Huang, T. S. Lorye, R. S. Dlott. Kaiser Permanente Regional Laboratories, Richmond, CA

Background: Serum TSH reference ranges are dependent on population characteristics, including prevalent thyroid disease and iodine status. Studies in the United States have demonstrated increasing TSH levels with age, and the American Thyroid Association recommends higher TSH goals for older patients on thyroid supplementation. Our objective was to establish TSH reference ranges in our racially diverse population in Northern California.

Methods: To gather TSH data from a healthy population, we used a retrospective strategy of database mining of our electronic medical records. We generated a report of all TSH results run in our laboratory from two weeks one year in the past. Only results from patients who were under our care for at least one year before the TSH test and continued care for the following year were included. Results were excluded from the reference population if the record of the patient noted thyroid-related disease or thyroid-related medication use at any time before or after the TSH test. Additional exclusion criteria included inpatient status at the time of collection and pregnancy during the two year period surrounding the TSH test. To obtain more data from our pediatric population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population, additional results from a 6-month period were added to our pediatric reference population.

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**Impact of alcohol consumption before thyroid evaluation**

G. Lima-Oliveira¹, A. C. P. Serra², F. E. P. Guimarães³, J. A. Corrêa⁴, R. F. F. Santos⁵, A. Souza⁶, D. F. B. Cavalcanti⁷, J. M. Moraes⁸, F. P. G. Santos⁹, M. D. R. Campelo⁹, G. C. Guidi¹⁰, G. L. Salvagno¹⁰, G. Lippi¹⁰.¹ University of Verona, Verona, Italy; ²PNÇ - Brazilian National Program of Quality Control, Rio de Janeiro, Brazil

**Background:** Patient related variables, such as physical exercise, stress and fasting status are important sources of variability in laboratory testing. However, clear instructions regarding alcohol consumption before thyroid evaluation is presently neglected by laboratory. This study aims to evaluate the impact of alcohol consumption on thyroid evaluation.

**Methods:** We studied 12 healthy volunteers at PNCQ (Brazilian National Program of Quality Control). A first blood sample was collected after an overnight fast (12 hours). Immediately after blood collection, the volunteers drank 50 mL (40% alcohol/volume) of Blended Scotch Whisky (Red Label, Johnnie Walker, Edinburgh, Scotland). Subsequent blood samples were collected at 1, 2, 4 and 6 hours after drank the Blended Scotch Whisky. Each phase of sample collection was carefully standardized, including the use of needles and vacuum tubes from the same lot. All thyroid evaluation was performed on the same analytical platform. The instrument was calibrated against appropriate proprietary reference standard material and verified with third-party control material (independent from calibrator material). Differences between samples were assessed by Wilcoxon ranked-pairs test. The level of statistical significance was set at P < 0.05.

**Results:** Main results are showed in Table 1.

**Table 1. Impact of alcohol consumption on thyroid evaluation**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Basal</th>
<th>1h</th>
<th>2h</th>
<th>4h</th>
<th>6h</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH μIU/ml</td>
<td>1.91 [1.32-2.26]</td>
<td>1.51</td>
<td>1.36</td>
<td>1.44</td>
<td>1.59</td>
</tr>
<tr>
<td>FT4 ng/dL</td>
<td>0.89 [0.79-0.96]</td>
<td>0.87</td>
<td>0.90</td>
<td>0.88</td>
<td>0.89</td>
</tr>
<tr>
<td>FT3 ng/dL</td>
<td>3.58 [3.83-4.09]</td>
<td>3.54</td>
<td>3.56</td>
<td>3.60</td>
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</tbody>
</table>

Conclusion: Alcohol consumption could jeopardize thyroid evaluation. Laboratory professionals should inform their customers to avoid alcohol consumption six hours (as minimal) before thyroid evaluation.

**A-225**

**Correlation and Performance of the SHBG, SDHEA and HCG assays between Chemiluminescence and Electrochemiluminescence Platforms**

C. O. W. Sabino, O. F. da Silva Filho. DASA, São Paulo, Brazil

**Background:** In reproductive assessment, monitoring of steroids are of uttermost importance to evaluate normalization of reproductive function. There are basic tests required for each procedure. To ensure the accuracy of the released results, it is important to ensure the correlation, precision and linearity of the tests used. This study
Tuesday, August 2, 9:30 am – 5:00 pm

Endocrinology/Hormones

5.4% 3.5%

®

67

37.3

2

10.0%

20.7

®

39.3

rd

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N

5.1%

148

2

3

Percent

49

20.1

N

21.7

8.6%

5.2%

85.8%

4.7%

Percent

20.0

2.2%

41.3


A-226


L. Heeren1, D. Dekker1, R. Schneider1, 1ProHealth Care, Waukesha, WI, 2Abbott Diagnostics, Abbott Park, IL

Background: Vitamin D testing has been consistently increasing over the previous 10 years. For many labs, the increase in volume justifies testing in-house. LC-MS-MS methods offer the advantage of accurately quantifying both the 25-hydroxy (OH) D2 and D3 levels, while antibody targeted methods such as those found on high throughput immunochemistry platforms do not delineate these two forms but rather provide a total 25(OH) level. To determine what the clinical impact would be when switching from our reference lab method (LC-MS-MS), patient data was collected during 2009 to evaluate D2, D3, and total vitamin D levels. These data helped influence our decision to bring the testing in-house. Patient data collection continued more recently to assess physician ordering and patient trend results.

Methods: An ad hoc report was generated for data analysis using Sunquest v. 7.1 and Ad Hoc Report Writer™. These data were originally collected in 2009. Yearly data collection continued through 2015. Data was processed for trends related to seasons, and frequency of result extremes indicating deficiency or possible toxicity. 25(OH) Total Vitamin D testing was performed on the Abbott ARCHITECT ci2000 system.

Results: Reference lab results indicated 86.7% of the patients tested in 2009, had undetectable vitamin D levels. Results obtained from correlation studies for SHBG comparing IMMULITE 2000 and ADVIA Centaur XP platforms, DHEAS and HCG assays on platforms ADVIA Centaur XP and Cobas E-170 shows statistical and clinical equivalence, corroborated by the analysis of the laboratory’s physicians.

A-227

Serum testosterone levels in status with diabetes and hypertension.

A. M. Jarrari1, S. Shakila2, W. Sherriff2, N. M. Jarari1, S. Panakala3, A. R. Said1, M. Y. Gounis1, S. D. Kolla1, R. Komreddy1, J. R. Peela2, M. G. Ellituri1, 1Faculty of Medicine, Bengahzi University, Bengahzi, Libyan Arab Jamahiriya, 2Faculty of Medicine, Quest International University Perak, IPOH, Malaysia, 3Mamata Medical College, Khammam, India, 1Department of Biochemistry, Rangya Medical College, NTR University of Health Sciences, Kakinada, India, 2Faculty of Medicine, Bengahzi University, Bengahzi, Libyan Arab Jamahiriya

Background: Hypogonadism in men is one of the conditions that need therapeutic attention and intervention. It is reported to be associated with aging, diabetes and statin therapy. Obesity, diabetes and statin therapy are common in Libyan subjects. There are few studies undertaken to study the prevalence, association with diabetes and statin therapy in Libyan subjects. Therefore the present study was carried out to evaluate and measure serum testosterone levels. Subjects on statin therapy were grouped further into two categories - those who are undergoing statin therapy less than one year and those more than one year.

Results: The serum testosterone level was comparatively lower in patients on statin therapy compared to the controls (p<0.05). There was no correlation between serum testosterone level with age, weight and waist circumference. The serum testosterone levels were lower in diabetic patients compared to the controls (p<0.05). The serum testosterone level is comparatively lower in diabetic patients on statin therapy for less than year (p<0.010). The diabetic patients on statin therapy for more than a year showed marked reduction in Serum testosterone levels (p<0.001).

Conclusion: There was significant reduction in serum testosterone levels in patients treated with statin therapy. The levels of serum testosterone were significantly reduced compared to control subjects. There was marked fall in serum testosterone levels in diabetic subjects which was further reduced by statin therapy. The presence of hypogonadism did not elicit greater fall in serum testosterone level in hypogonadists or diabetics with hypertension.

A-228

Evaluation of Access TSH (3rd IS) Assay with Comparison to Multiple Platforms

S. P. Wyness1, J. A. Strasser2, L. Prust1, P. Nunnelly1, R. M. Ruiz2, R. H. Christensen1, 1 ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, 2Department of Pathology, University of Utah, Salt Lake City, UT, 3Beckman Coulter, Inc., Chaska, MN, 4University of Maryland, Baltimore, MD

Objective: Standardization of TSH assays has been a concern for several years. Beckman Coulter has developed a new Access TSH (3rd IS) assay. Method comparison was performed with the new Access TSH (3rd IS) assay and five commercially available TSH assays.

Method: 156 frozen serum samples with TSH values between 0.011 and 41.711 mU/L were tested using Beckman Coulter Access TSH (3rd IS), Access HYPERSensitive I SH and Access Fast I SH, Roche cobas® e602 TSH, Siemens ADVIA Centaur® XP TSH Ultra, and Abbott ARCHITECT® TSH assays. All samples were tested in singleton by two clinical laboratories. Method comparisons were performed following CLSI EP09-A3 guidelines. Passing-Bablok regression analysis was conducted using ongoing discussions about how to define healthy or toxic vitamin D levels, this study suggests that the majority of patients are maintaining adequate vitamin D levels throughout the seasons.

S64

Access TSH (3rd IS) as the comparison method. Two-sided 95% confidence intervals were reported on slopes, intercepts and predicted biases at clinically relevant TSH concentrations of 0.4 and 4 µIU/mL.

Results: Method Comparison results presented in Table 1.

Conclusion: Method comparison biases varied significantly between TSH assays and manufacturers. This is consistent with literature and supports efforts to harmonize TSH amongst manufacturers.

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<table>
<thead>
<tr>
<th>Access TSH (3rd IS) Method</th>
<th>Intercept [95% CI]</th>
<th>Slope [95% CI]</th>
<th>Predicted bias at 0.4 µIU/mL</th>
<th>Predicted bias at 4.0 µIU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Access hTSH</td>
<td>[0.97 ± 1.00]</td>
<td>[0.94 – 1.09]</td>
<td>0.97</td>
<td>-0.05 (13.6%) – 0.03 (13.6%)</td>
</tr>
<tr>
<td>Access Fast hTSH</td>
<td>[0.91 ± 0.94]</td>
<td>[0.84 – 0.97]</td>
<td>0.98</td>
<td>-0.02 (6.2%) – 0.00 (6.2%)</td>
</tr>
<tr>
<td>Cobas TSH</td>
<td>[0.94 ± 0.98]</td>
<td>[0.86 – 0.96]</td>
<td>0.98</td>
<td>-0.08 (20.9%) – 0.00 (20.9%)</td>
</tr>
<tr>
<td>Centaur TSH</td>
<td>[0.90 ± 0.93]</td>
<td>[0.80 – 0.94]</td>
<td>0.99</td>
<td>-0.03 (10.0%) – 0.00 (10.0%)</td>
</tr>
<tr>
<td>ARCHITECT TSH</td>
<td>[1.19 ± 1.21]</td>
<td>[1.17 – 1.21]</td>
<td>0.99</td>
<td>0.04 (9.7%) – 0.02 (9.7%)</td>
</tr>
</tbody>
</table>

**A-229**

Impact of metformin in Thyroid Stimulating Hormone (TSH) in elderly persons with diabetes mellitus

R. Fontes¹, P. F. Teixeira², O. Fernandes², M. D. Freire², Y. Schrank², P. B. C. Araujo¹, E. M. R. Cavallari¹, I. Bender², M. C. Pinheiro¹, D. M. Gomes², M. Vaisman². ¹DASA/Universidade Federal do Rio de Janeiro, RIO DE JANEIRO,RJ, Brazil, ²Universidade Federal do Rio de Janeiro (UFRJ), RIO DE JANEIRO,RJ, Brazil, ³DASA, RIO DE JANEIRO, RJ, Brazil

Background: Studies have reported higher prevalence of hypothyroidism in persons with diabetes mellitus (DM). Metformin (MTF), the most widely used drug to treat DM, reportedly interfere with TSH levels. The aim of this study was to evaluate the influence of MTF on serum TSH. Methods: 633 elderly were studied, 224 with DM in use of MTF, (MTF patients), compared to 409 non-DM (NDM) with undiagnosed hypothydoidism without levothyroxine use. A statistical model was used providing slopes, intercepts and predicted biases at clinically relevant TSH concentrations of 0.4 and 4 µIU/mL.

**A-230**

Evaluation of the Testosterone II Assay* on the ADVIA Centaur System

N. Parker¹, K. Thakur¹, S. Pagliaro³, T. Spence². ¹Siemens Healthcare Diagnostics, Tarrytown, NY, ²Siemens Healthcare Diagnostics, Newark, DE

Background: Testosterone (4 androsten 17β-ol-3-one) is a C19 steroid hormone with a molecular weight of 288.4 daltons. Testosterone is the major androgen in males and is controlled by luteinizing hormone (LH). LH is released from the anterior pituitary exerting the primary control on testosterone production and acting directly on the Leydig cells in the testes. Testosterone stimulates adult maturation of external genitalia and secondary sex organs, and the growth of beard, axillary, and pubic hair. Siemens has developed an improved testosterone assay with acceptable sensitivity and precision to be able to measure both adults and pediatric males and females. This is an 18-minute competitive immunoassay with an assay range of 7.0 to 1500 ng/dL. The assay is aligned to the CDC HoSt standardization program.

Method: The alignment to the CDC HoSt was achieved by running 40 serum samples with ID-LCMS/MS Testosterone RMP assigned values on 3 lots of ADVIA Centaur® Testosterone II reagents and calibrators. Ten (10) standards were value assigned by fitting the raw (RLU relative light units) data obtained from the ADVIA Centaur instrument independently for each lot. The method comparison equation closest to an r value equal to 1.0 was selected. The equation was solved for the RLU associated with the 10 standards. The assay’s performance was assessed by measuring 3 reagent lots for imprecision and functional sensitivity which were evaluated by assaying control materials and serum pools twice a day for 20 days, for a total of 80 replicates. A method comparison to the CDC HoSt Testosterone reference method was assessed using 1 lot of reagent and 128 serum samples. Reference ranges were also generated for adult and pediatric males and females.

Results: The data obtained with the ADVIA Centaur Testosterone II assay demonstrated good correlation to the ID LC/MS/MS Testosterone RMP yielding a Passing-Bablok slope of 0.97, intercept of + 1.94 ng/dL, and regression coefficient of 0.98. A 20 day precision study yielded within lab precision CV’s of between 3.0% and 8.4% for the 3 lots using samples between ~25.0 ng/dL to ~1120.0 ng/dL of testosterone for the assay. The functional sensitivity for all 3 lots was < 7.00 ng/dL. Reference ranges (median and 95% confidence intervals) for both male and female adults and pediatrics (ages and Tanner stages) have been generated.

Conclusion: The Siemens ADVIA Centaur Testosterone II assay alignment with the CDC HoSt should be a valuable tool in clinical laboratories for the accurate measurement of testosterone in human sera.

* Under FDA review. Not available for sale. Product availability will vary by country.
Tuesday, August 2, 9:30 am – 5:00 pm

Population study of TSH Variant in Brazilian population

C. O. W. Sabino, C. O. W. Sabino, D. Waltrick, O. F. da Silva Filho. DASA, São Paulo, Brazil

Background: The Thyroid Stimulating Hormone (TSH) is a hormone formed by two chains: alpha and beta polypeptide. Released by the pituitary through hypothalamic TRH action TSH interacts with a specific cell receptors in the surface of the thyroid cell applying two main functions: To stimulate cells and hypothyroid reproduction, and to stimulate thyroid gland on synthesizing and secreting T3 and T4. Thus, TSH becomes the main regulator of thyroid function and best indicator of discrete changes in the production of thyroid hormones. First generation assays allow the diagnosis of hyperthyroidism, second and third generation assays increase the diagnostic certainty and it is possible to be used also for the detection of hyperthyroidism since it has a sensitivity and specificity of 96% and 93% respectively. A study in California with 1,611 million patients identified a rare variant of TSH in which the monoclonal antibodies used in the 3rd generation tests failed and resulted in a falsely low value, this variant was found in a small percentage of the population with descendants of South Asia. This study aims to investigate the presence of this variant of TSH in the Brazilian population.

Materials and Methods: A total of 329,175 samples were tested according to the manufacturer’s instructions for the TSH3-UL assay (Siemens Healthcare Diagnostics) in ADVIA Centaur System (Siemens Healthcare Diagnostics). Within those samples, 1,508 had TSH <0.01 U/L of which 704 were evaluated. 804 samples were not evaluated because they failed to pass the minimum TSH threshold needed for interpretation. To confirm the presence of the variant, TSH and BioT were measured in TSH-2 assay (Siemens Healthcare Diagnostics, ref 0.35 to 5.50 ref U/L) and TSH-3-UL (Siemens Healthcare Diagnostics, ref 0.55 - 4.78 ref U/L), all presented results <0.01 showing complete agreement between the methods. This study shows that TSH variant was not found in the studied population. In addition, the California study showed that the variant was found in people of South Asian descendants, and according to data from IBGE 2010 Brazil does not have immigrants of this region but from East Asia, suggesting the potential non-existence of carriers of this mutation in the Brazilian population.

Investigation of posture specific reference intervals for plasma metanephrine and normetanephrine

J. M. Boyd1, G. Kline2, H. Sadzadeh1. 1Calgary Laboratory Services, Calgary, AB, Canada, 2University of Calgary, Calgary, AB, Canada

Introduction: Metanephrines, the metabolites of catecholamines, are tested in the investigation and workup of pheochromocytoma and paraganglioma (PPGL). In the majority of cases of PPGL, plasma metanephrine (PM) and/or normetanephrine (PNM) are elevated at least 2 times the upper limit of normal. Like many other tests, PM and PNM are affected by many preanalytical variables such as diet, drugs, exercise and stress that may result in a slightly elevated result in the absence of PPGL. Also, several studies have recently shown that collection posture is a significant preanalytical interference as patients without PPGL have lower PM and PNM if they are supine for 30 min prior to phlebotomy. The 2014 PPGL guidelines from the American Endocrine Society recommend supine collection for plasma metanephrines to reduce the number of false positive results. However, most laboratories do not require supine collection for plasma metanephrines and do not provide a posture specific reference range. In this study, we investigated our laboratory population to determine the performance of our current reference intervals with and without collection posture specifications.

Materials & Methods: All PM and PNM results between May 2010 and September 2015 were requested from our laboratory information system. Patient information was deidentified and the final data set obtained included the numerical metanephrines result, order date, and collection location. Data was analyzed using Microsoft Excel 2007 into histograms displaying the distribution of patient results and was compared to the current reference intervals of <0.5 mmol/L for metanephrine and <0.9 mmol/L for normetanephrine.

Results: The data pull resulted in 5,452 plasma metanephrines results from 5,068 patients. All specimens were assumed to be collected in the seated position (as indicated in our SOP), with the exception of samples collected at our local endocrinology testing unit which is known to collect after 30 min in the supine position. This subgroup consisted of 313 specimens on 269 patients and was analyzed separately. No effect of posture was observed between PM collected in the supine and seated position; epinephrine, the precursor for PM, is an adrenal catecholamine and should not change with posture. However, the distribution of PNM collected in the seated position had a large tail that extended over the upper limit of normal (0.9 ng/ mL), resulting in 14% of all patients between 0.9 and 1.8 mmol/L. Only 7% of patients collected in the supine position fell into this range, which shows the clear effect of posture. Norepinephrine, the precursor for PNM, is secreted from both the CNS and is expected to change with posture.

Conclusion: Collection of plasma metanephrines in the seated position can lead to a significant number of slightly elevated PNM results in patients who do not have PPGL. Investigation of posture specific reference ranges should be considered.

Validity of Free and Bioavailable Testosterone Calculations Using Abbott Architect Total Testosterone and Sex Hormone Binding Globulin Immunoassay Results

D. E. Palmer-Toy1, C. Tabata1, O. C. Hszieh1, D. W. Chandler1, K. Chun1, B. Holmquist1, Kaiser Permanente - Southern California Permanente Medical Group, North Hollywood, CA, 2Endocrine Sciences - LabCorp, Calabasas, CA

Objective: Comparison of directly-measured and calculated free (fT) and bioavailable testosterone (BioT) concentrations.

Background: In the diagnosis of testosterone deficiency in men and excess in women, clinicians often rely upon calculated fT and BioT derived from web-calculators. The accuracy of such calculations should be formally validated for the specific immunoassays employed.

Methodology: Sera from thirty seven patients were used, including a mixture of men and women. Measurement of fT and BioT was performed using tandem mass spectrometry after equilibrium dialysis or ammonium sulfate precipitation, respectively. Abbott Architect total testosterone (T) and sex hormone binding globulin (SHBG) immunoassay results were used in fT and BioT calculations applying the Vermeulen formulae with measured (bromcresol green and purple) or fixed (4.3 g/dL) albumin concentrations. The Abbott SHBG assay was recalibrated after completion of our measurements, so fT and BioT were calculated using both SHBG results with the former calibration and after numeric adjustment for the new calibration: SHBG recalibrated = 0.94 * SHBG (old calibration) - 0.2

Results: Concentration span: T (LC/MS) 7 - 1287 ng/dL; SHBG (Abbott) 18 - 89 mmol/L; fT (LC/MS) 0.5 - 284 pg/mL; BioT (LC/MS) 1 - 952 ng/dL.

Correlation studies:

(1) T (Abbott) = 1.14 * T (LC/MS) - 22.6; R2 = 0.989
(2) fT (Calc) = 1.53 * fT (LC/MS) + 0.58; R2 = 0.952 [old SHBG calibration; Alb = 4.3 g/dL]
(3) fT (Calc) = 1.30 * fT (LC/MS) + 0.25; R2 = 0.981 [SHBG recalibration; Alb = 4.3 g/dL]
(4) BioT(Calc) = 1.11 * BioT (LC/MS) + 4.2; R2 = 0.932 [old SHBG calibration; Alb = 4.3 g/dL]
(5) BioT(Calc) = 1.14 * BioT (LC/MS) + 4.72; R2 = 0.924 [SHBG recalibration; Alb = 4.3 g/dL]

Calculated fT and BioT with fixed or BCP or BCG measured albumin did not differ significantly.

Qualitatively, there was significant dispersion among BioT results in the crucial range of 150 - 450 ng/dL with or without SHBG recalibration.

Conclusions:

1. fT results calculated from Abbott Architect T and SHBG results and the Vermeulen formula with fixed albumin concentration correlate well, but do not correspond to directly measured results. Transformed reference ranges should be transferrable.
2. SHBG recalibration has improved the fT correlation
3. BioT results calculated from Abbott Architect T and SHBG results and the Vermeulen formula did not correlate well across the typical clinical decision range.
4. SHBG recalibration did not improve the BioT correlation.
5. Use of measured rather than fixed albumin concentration did not significantly alter the fT or BioT correlation.
A Sudden Manufacturer Discontinuation Makes Free Testosterone Testing Quite Testy

A. J. McShane, R. Kreller, M. Strizzi, S. Wang. Cleveland Clinic, Cleveland, OH

Introduction: Testosterone is a hormone that stimulates the development of male genital and secondary sex characteristics. In women, it has a role as an estrogen precursor. Elevated testosterone can lead to premature puberty in males or virilization in females. Conversely, decreased testosterone may lead to hypogonadism in males and decreased libido in females. Testosterone is primarily found tightly bound to sex hormone binding globulin (SHBG) and loosely bound to albumin in blood. Measurement of the free fraction is indicated in patients with abnormal SHBG. It can be obtained via equilibrium dialysis or ultrafiltration. In our laboratory, radioactive (tritium-labeled) testosterone is added to the patient’s serum. After equilibration, the sample is filtered through a low molecular weight cut-off filter which allows the free testosterone to pass through. The activity of the retentate and filtrate is then measured to determine the ratio of free over total testosterone. This is then multiplied by the total testosterone to achieve the free testosterone levels. Our commercially purchased, tritiated testosterone is purified via column chromatography before use to remove potential radioactive impurities. However, the commercial provider of the column is discontinuing its production. Three alternatives to the current chromatography were proposed for this high-volume test: in-house column assembly, a new column manufacturer, and forgiving column purification. The first option was abandoned because of potential differences in column-to-column performance, without strict quality control parameters. The latter options were evaluated to determine an acceptable replacement for the discontinued columns. Methods: Twenty-six patients were compared between the current and potential columns. An additional 26 patients were compared between the current columns and a column-less sample preparation. We further compared the column-less approach with the current columns using 100 additional samples (50 males and 50 females). Results: In the current versus potential column study, Deming regression of the free testosterone levels revealed a slope of 0.926, a correlation coefficient of 0.9929, and a mean percent bias of -6.19%. The column-less comparison gave a slope of 1.405, a correlation coefficient of 0.9772, and a free testosterone mean bias of +33.76% affirming that a freely-filtered, radioactive impurity exists in our commercial radioactive testosterone. The regression formula from the current column versus column-less methodologies was used to correct this bias, and applied to the 100 sample study. The Deming regression comparison of the 100 patients, after the correction, gave a slope of 0.914, a correlation coefficient of 0.9872, and a mean percent bias of -4.27%. Conclusion: A new column-less technique gave similar results (bias <5%) for free testosterone levels, to the discontinued commercial columns. The column-less technique requires a correction; however, it has the added benefit of requiring less radioactive material and significantly reducing technician time.

Establishing a clinical cutoff for aldosterone-direct renin concentration ratio using retrospective data

D. Orton1, A. Leung2, G. Kline1, A. Chiu1. ‘Calgary Laboratory Services, Calgary, AB, Canada. 1University of Calgary Cumming School of Medicine, Calgary, AB, Canada

Objective: To characterize aldosterone-direct renin concentration ratio (DRC-ARR) ranges appropriate for evaluation of primary aldosteronism using population data for validated plasma renin activity ARR (PRA-ARR) as a benchmark. Relevance: The ARR is the standard screening test for primary aldosteronism. Past methods used to derive the ARR mainly employed the PRA, but with increased workloads and automation capabilities, the DRC has become more widespread. However, studies focusing on appropriate ARR cutoffs for primary aldosteronism using DRC are lacking. Methodology: Data was obtained from Calgary Laboratory Services from January, 2010 to December, 2015. Indirect comparison of the performance of DRC-based ARR and PRA thresholds was assessed using ROC curve analysis analyses using PRA-ARR values as the gold standard. Renin levels from each method were also evaluated for classification of “low renin” status. Results: Results from 5864 patients were obtained (associated with 6074 PRA-ARRs and 1405 DRC-ARRs). The Canadian Hypertension Education Program PRA-ARR threshold of >550 pmol/L/ng/mL showed a prevalence of PA 37.7%. Within the elevated PRA-ARR patients, “low renin” was defined as the 95th percentile of the renin values obtained (<2.05ng/mL/h). Using a DRC-ARR value of >25 pmol/L/mIU/L the prevalence was 38.9% and “low renin” was <28.0 mIU/L. Employing these low renin values for prediction of elevated ARR demonstrated sensitivities and specificities of 95% and 49% for PRA <2.05 ng/mL and 95% and 56% for DRC <28.0 mIU/L. Conclusions: A DRC-ARR threshold of >25 pmol/L/mIU/L performed with similar high sensitivity to the conventional PRA-ARR threshold of >550 pmol/L/mg/mL for PA. Additionally, DRC levels <28.0 mIU/L should be considered compatible with low-renin status in a hypertension population. Retrospective assessment of laboratory data is a useful tool to establish reference intervals and cutoff for clinical management of patients.
Frequency of Pseudohyper- and Pseudohypo-calcemia in an Emergency Department Setting at a large Tertiary Care Hospital

S. Roychoudhury, M. Solanki, L. Bilello, S. Weinerman, L. K. Bjornson
North Shore University Hospital, Northwell Hofstra School of Medicine, Manhasset, NY

Introduction: The total serum/plasma calcium (Ca) concentration consists of three fractions with approximately 15% bound to organic & inorganic anions, 40% bound to albumin and 45% containing the physiologically active “ionized calcium” or more accurately the free & unbound calcium (CaIO). Total calcium (Ca) is part of the basic metabolic panel and is most often used as the primary indicator of calcium status because the assay is easily automated, rapid & cost effective. However, the CaIO (free or ionized calcium) concentration is considered to be a more accurate indicator of calcium status since it is the physiologically active fraction & is tightly regulated by parathyroid hormone & vitamin D. The concordance between total & ionized calcium measurements is moderate in healthy subjects and is poor in patients with severe acid-base disorders, hyperparathyroidism, hyperphosphatemia, or chronic kidney disease. Total calcium concentrations can change independently of ionized calcium concentrations and vice versa. If the Ca concentration is elevated in the setting of a normal CaIO, it is termed pseudohypercalcemia., and if Ca is low with a normal CaIO, it is termed pseudohypocalcemia.

Objective / Methods: This study was designed to determine the frequency of pseudohyper- and pseudohypo-calcemia and to calculate concordance of Ca & CaIO measurements from patients in an Emergency Department setting. Ca measurements were performed on the Roche Modular P800 system using 0-cresol-phthalein complexone and CaIO measurements performed on the Radiometer 827 blood gas system using ISE (ion selective electrode) methodology.

Results/Discussion: A computer search of the data base from the E. D. of a large tertiary care hospital in Long Island, NY identified 334 patients with paired Ca & CaIO results from specimens drawn within 1 hour on each patient. No clinical history or other lab results were considered for this study. Based on the Ca concentration (reference range 8.4 - 10.5 mg/dL), 7 out of 334 patients (2.1%) were classified hypercalcemic, 307 (91.9%) normocalcemic and 20 (6.0%) hypocalcemic. In the hypercalcemic group, 2 of 7 patients had normal CaIO results (reference range 1.12 - 1.30 mmol/L) and were classified as pseudohypercalcemic (0.6% of total group / 28.6% of hypercalcemic group). In the hypocalcemic group 14 of 20 patients had normal CaIO results and were classified as pseudohypocalcemic (4.2% of total group / 70% of hypocalcemic group). Additionally, in the 307 patients classified as normocalcemic by Ca, 36 had abnormal CaIO concentrations (29 low & 7 high CaIO) showing an 88.3% concordance between Ca & CaIO.

Conclusion: The frequency of pseudohypercalcemia is very low in the whole group (0.6%) but was 28.6% for the hypercalcemic group. The frequency of pseudohypocalcemia was higher in the whole group (4.2%) and much higher (70%) in the hypocalcemic group. Concordance of Ca & CaIO in the normocalcemic group was 88.3%. These results indicate that ionized calcium measurements should be considered, especially in hypo- and hyper-calcemia classified by Ca concentrations.