Tuesday, July 28, 2015

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

Endocrinology/Hormones

A-066

Development and Performance of a LOCI Testosterone Assay on the Dimension Vista System

T. Gorzynski, S. Crawford, M. Wasson, S. Lewisch, Z. Teng, M. Drinan. *Siemens Healthcare Diagnostics Inc., Newark, DE*

We describe a fully automated homogeneous competitive binding immunoassay for total testosterone (TTST)* on the Dimension Vista® System based upon LOCI® technology. The TTST method utilizes three reagents: two synthetic bead reagents and a biotinylated monoclonal antibody specific for testosterone. The first bead reagent (Sensibeads) is coated with streptavidin and contains photosensitizer dye. The second bead reagent (Chemibeads) is coated with a testosterone analog and contains chemiluminescent dye. Sample is reacted with a displacer to release testosterone bound to endogenous sex hormone binding globulin, which then competes with Chemibeads for biotinylated antibody to form bead-analog-antibody immunocomplexes. Addition of Sensibeads leads to formation of bead pair complexes. Illumination of the complex by light at 680 nm generates singlet oxygen from Sensibeads, which diffuses into Chemibeads to trigger a chemiluminescent reaction. The resulting signal (measured at 612 nm) is directly related to analyte concentration. The assay uses a 10 µL sample volume of serum or plasma and has an analytical range of 8-1000 ng/dL undiluted. With dilution, samples up to 2000 ng/dL can be tested. Results are traceable to the CDC ID-LC-MS/MS reference method. Time to first result is 23 minutes. Precision was evaluated per CLSI EP5 using serum pools and commercial quality control materials. Repeatability and within-lab precision were < 4.9 %CV and < 7.0 %CV, respectively, across the assay range. Good agreement was observed in patient sample method comparison studies versus two different systems: Dimension Vista = 0.93 * ID-LC-MS/MS + 3.9 ng/dL (r = 0.99, n = 38), Dimension Vista = 0.90 * Roche ELECSYS® - 2.60 ng/dL (r = 0.99, n = 215). Minimal cross reactivity (< 10%) was observed with key compounds including: androstenedione, androsterone, 5a-dihydrotestosterone, corticosterone, 11-deoxycortisol, DHEA, DHEA-sulfate, 17b-estradiol, progesterone, cortisol, dexamethazone, danazol, 17a-methyltestosterone, 11b-hydroxytestosterone, and 11-ketotestosterone. **Conclusions.** We conclude that Dimension Vista Testosterone Assay with LOCI technology provides acceptable sensitivity, precision, accuracy, turnaround time, and dynamic range, and shows a high level of agreement with the Testosterone assays on Roche Elecsys and ID-LC-MS/M. *Product under development-Not available for sale

A-067

Hair cortisol as a biomarker of the HPA axis in pregnant women with asthma

L. Smy[1], K. Shaw[2], B. Carleton[2], G. Koren[1]. *[1]The Hospital for Sick Children / U of T Leslie Dan Faculty of Pharmacy, Toronto, ON, Canada, [2]Child & Family Research Institute / BC Children's Hospital / UBC, Vancouver, BC, Canada*

Background: Hair cortisol analysis has been used to assess the effect on the hypothalamus-pituitary-adrenal (HPA) axis by a variety of psychiatric and physical stressors. Because hair grows on average 1 cm/mo, such analysis allows detecting changes over time. For patients with Cushing syndrome, the hair cortisol level paralleled the high endogenous levels due to the disease, and it is 86% sensitive and 98% specific for the detection of cycling Cushing syndrome. Recently, we observed that hair cortisol of children with asthma was two-fold lower when taking inhaled corticosteroid than prior to the medication. Due to the importance of cortisol in the fetal maturation process and the stress response, the objective was to examine whether hair cortisol is a sensitive biomarker to assess the effects of asthma on the HPA axis in pregnant women.

Methods: A prospective case-controlled study was carried out to collect hair samples from pregnant women with and without asthma. Women were eligible to participate if they did not use topical corticosteroids on their scalp or have a pre-existing condition characterized by high cortisol levels. Hair samples were segmented to provide cortisol results corresponding to pre-conception (PC), trimesters 1-3 (T1-3), and post-partum (PP) based on the average growth rate of 1 cm/month. The hair cortisol levels were measured using a validated ELISA method (Spearman rho = 0.92, $p < 0.0001$, when compared to a hair cortisol LC-MS methods). The intra- and inter-day coefficients of variation were 1.7% and 2.5%, respectively. The hair cortisol results were compared within and between the two groups of women using the appropriate parametric or non-parametric tests.

Results: Hair samples for 93 pregnant women, 31 without asthma and 62 with asthma, were analyzed thus far. In healthy controls, there was an increase in hair cortisol over the course of pregnancy, with statistically significant changes occurring up to T2 or T3. This trend, also shown by other groups, was dampened in women with asthma. Women with asthma had significantly lower hair cortisol levels in T2 and T3 ($AUC_{T2} = 0.664 \pm 0.071$ (SEM), $p = 0.03$; $AUC_{T3} = 0.795 \pm 0.088$ (SEM), $p = 0.01$).

Conclusions: Hair cortisol successfully detected the expected increase during the course of a healthy pregnancy. In contrast, asthma was associated with a diminished ability to increase cortisol levels in late pregnancy. Children born to women with asthma have been found to have an increase in congenital malformations, endocrine or metabolic disorders, and digestive system diseases. Future research will need to establish the potential role of the changes in cortisol in pregnancy on pregnancy outcomes and fetal well-being.

A-068

Screen with Reflex to Better Test Utilization: A Cost Analysis of Thyroglobulin Testing Strategies

J. L. Powers, F. G. Strathmann, J. A. Straseski. *University of Utah, Salt Lake City, UT*

Determination of thyroglobulin (Tg) concentration is important for monitoring recurrence of thyroid cancer. In traditional immunoassay detection of Tg, accurate quantitation may be affected if Tg antibodies (TgAb) are present in the patient sample. Quantitation of Tg by liquid chromatography – tandem mass spectrometry (LC-MS/MS) accurately measures Tg in TgAb-positive patients by eliminating this possible interference. Typically, Tg and TgAb quantitation can be ordered separately or as part of a reflex in which TgAb status is determined first. In reflex testing, if the patient sample is negative for TgAb, then Tg is quantified using an immunoassay; if positive, Tg is determined using LC-MS/MS. This may have important implications for overall cost since immunoassay testing is often less expensive than LC-MS/MS analysis. To examine this we reviewed ordering patterns for almost 100,000 unique patients after reflex testing became available in our laboratory. Cost analysis was performed using an average of list prices from three different laboratories. For this data set, 90,312 orders for reflex testing occurred compared to 11,279 separate orders for Tg by LC-MS/MS. From the reflex testing subset, 11% of the samples were TgAb-positive and therefore reflexed to LC-MS/MS for Tg quantitation. For TgAb-negative samples, the reflex to immunoassay for Tg quantitation resulted in a total cost savings of over $9 million compared to the cost if LC-MS/MS were utilized when not required for accurate quantitation. There were also 49,018 standalone orders for TgAb, of which approximately 5% also ordered Tg by LC-MS/MS. In this subset, 10% of samples were positive for TgAb, showing good agreement with the percent TgAb-positives seen in the reflex testing subset. Of this group, 64% represented separate orders on the same date. It is possible these clinicians were unaware of the reflex testing option offered by the laboratory. Since the overwhelming majority (90%) of samples in this subset were TgAb-negative, had these samples been ordered as part of a reflex test and sent to immunoassay for Tg quantitation, a total savings of almost $300,000 could have been realized. For patients known to be TgAb-positive, reflex testing is unnecessary and Tg is best quantified using LC-MS/MS. In the subset of separately ordered TgAb and Tg by LC-MS/MS with a previously known TgAb result, 12% were TgAb-positive. Less than half of these samples represented TgAb results obtained within the past six months. In summary, the majority of clinicians utilized the reflex testing option for Tg which resulted in tremendous cost savings since, according to our data, only approximately 10% of patients were TgAb-positive. A smaller percentage of clinicians ordered TgAb and Tg separately by LC-MS/MS when the antibody status was either unknown (ordered the same day) or was previously determined to be negative, resulting in unnecessary utilization of the more expensive LC-MS/MS testing. These data provide a current example that identifying situations in which using more expensive testing methods (*e.g.* LC-MS/MS) is most appropriate leads to more economical use of valuable health care resources.

A-069

Characterization of indirect effect of canagliflozin (Invokana), a sodium-glucose cotransporter 2 inhibitor, to inhibit reabsorption of 1,5-anhydroglucitol by sodium-glucose cotransporter 4

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

Background: Renal reabsorption of 1,5-anhydroglucitol (AG), a non-metabolizable glucose analogue acquired from diet, is competitively inhibited by elevated glucose, often leading to a depleted state in diabetes. Recovery of plasma AG from a depleted state correlates with improved glycemic control. Drugs that inhibit glucose reabsorption in the kidney (sodium-glucose cotransporter 2 (SGLT2) inhibitors) can negate this correlation, however, because they also promote a decrease in AG. The mechanism is indirect: whereas AG reabsorption occurs via SGLT4, inhibition of SGLT2 elevates urine glucose which in turn competitively inhibits AG reabsorption by SGLT4. Using literature data for the effect of the SGLT2 inhibitor canagliflozin to decrease AG, our objective was to quantitatively characterize the effect on AG reabsorption, and therewith to estimate the corresponding half-life (t1/2) for changes in AG upon initiation of canagliflozin therapy (CT). The utility of t1/2 characterization is that AG measurements might serve as an early adjunct marker for canagliflozin activity.

Methods: Primary data (Balis et al., J Diabetes 2014;6:378-80) were serum AG concentrations ([AG]) 26 weeks post initiation of CT (300 mg/day): [AG] = 1.4 ± 0.7 µg/mL (reference range: [AG] = 7.2-33.3 µg/mL). Analysis used an established two-compartment AG mass balance model (Am J Physiol Endocrinol Metab 1997;273:E821-E830). Changes in total body mass of AG (T) reflect any difference between ingestion and excretion rates: $dT/dt = ki - \alpha T$ (Eqn.1), where ki = AG ingestion rate (mg/day), and $\alpha = GFR (1-f)/(1+K)/V$, where GFR = glomerular filtration rate (mL/min), K is the ratio between tissue and plasma compartments (K = 2.1), V is the plasma volume (nominally 3 L), and f is the fractional reabsorption in kidney of filtered AG (in normoglycemia, f = 0.9984). For steady-state (ingestion rate = excretion rate), ki = [AG] GFR (1-f) (Eqn.2). Normal distributions for ki (4.62 ± 1.62 mg/day) and GFR (80-120 mL/min) are known. Assuming that model parameters other than f are unaffected by CT, then values for f in CT are computed from Eqn.2 using the [AG] values reported for CT patients. Determination of f then allows calculation of the expected t1/2 for changes in [AG] upon initiation of CT (derived from Eqn.1): $t1/2 = -\ln(0.5)/\alpha$.

Results: The distribution [AG] = 1.4 ± 0.7 µg/mL (average ± 1sd) observed in CT patients is consistent with average f = 0.977 (range: f = 0.984-0.953). Compared to normoglycemia (f = 0.9984), the decrease in f was <5%. CT nonetheless decimates [AG] relative to the reference range ([AG] = 7.2-33.3 µg/mL). Decrease in [AG] post CT therapy is predicted to be rapid, with average t1/2 = 1.95 days (range: 2.8-0.95 days).

Conclusions: According to model calculations, modest decreases (<5%) in fractional reabsorption of AG account for the drastic decrease in [AG] observed during CT. Decreases are predicted to be rapid (t1/2 < 3 days) after initiation of CT. Although CT negates the usual premise of AG measurement (that [AG] should increase with improved glycemic control), the rapid effect on [AG] means that AG measurement could instead provide an early independent measure of effective CT.

A-070

Development of quantitative Progesterone assay for fully automated analyzer LUMIPULSE® G1200

Y. Kitamura, K. Umeda, S. Ando, K. Aoyagi. *Fujirebio.Inc, Tokyo, Japan*

Background: Progesterone (PRG) is one of an endogenous steroid hormone involved in the menstrual cycle, pregnancy, and embryogenesis. It is generally used to confirm the existence of ovulation and the corpus luteum function. In this time, we developed new reagent (Lumipulse PRG-N) which has excellent correlation with ID-GC/MS and reference materials and improved cross reactivity to some drugs or PRG derivative. Lumipulse PRG-N is one-step immunoassay, and PRG in specimen samples and PRG coated on the micro particle competitively react with ALP labeled anti PRG antibody. It is finally detected based on CLEIA technology. Here we show the excellent fundamental performance with fully automated chemiluminescence analyzer LUMIPULSE G1200.

Methods: The sample types used for this study were serum or heparin Li-plasma. Correlation with ID-GC/MS, commercial competitive kit, matched pair correlation between serum and plasma, cross-reactivity to drugs, within-run and between day precision, limit of quantification (LoQ) were evaluated following recommendation from CLSI documents EP-5, EP-7, EP-12, EP-14 and EP-17. All evaluations were executed with LUMIPULSE G1200 (FUJIREBIO INC.).

Results:

Correlation with ID-GC/MS using 40 specimen samples was excellent (slope: 1.02, regression: 0.98) and the measurement value in Lumipulse PRG-N calibrators was traceable to two different kinds of reference materials (ERM-DA377 and BCR348R). The significant correlation with the commercial available kit with 79 specimen samples was observed (Cobas, slope: 0.85, regression: 0.991, ARRCHITECT, slope: 0.98, regression: 0.983). Correlation between serum and heparin Li-plasma with 60 matched pair samples was excellent (slope: 1.05, regression: 0.997). Within-run and between day precision % CVs for our assay ranged from 2 to 5% when 3 different conc. of samples were tested, LoQ was calculated at 0.17 ng/mL by precision profile. As a result of evaluation with total 19 kinds of drugs and PRG derivatives, significant cross reactivity with almost all cross reactants were not observed.

Conclusion: These results demonstrated that Lumipulse PRG-N was a precise and highly useful for measuring PRG in serum and heparin Li-plasma. Also this assay is perfectly traceable to ID-GC/MS and reference materials.

A-071

Pediatric and Adult Reference Intervals for Key Endocrine and Special Chemistry Markers based on the Canadian Health Measures Survey (CHMS)

M. Nieuwesteeg[1], V. Higgins[1], J. E. Raizman[1], Y. Chen[1], S. L. Wong[2], D. Blais[2], K. Adeli[1]. *[1]Hospital for Sick Children, Toronto, ON, Canada, [2]CHMS Program, Statistics Canada, Ottawa, ON, Canada*

Background: Accurate, up-to-date reference intervals are essential for correct interpretation of clinical laboratory test results. Concentrations of circulating biomarkers can vary profoundly with age and gender, making it essential that reference intervals are stratified accordingly. The Canadian Laboratory Initiative for Pediatric Reference Intervals [CALIPER] has been establishing a new reference interval database for pediatric disease biomarkers, to address the current gaps in pediatric reference intervals. Recently, CALIPER has collaborated with Statistics Canada to access data obtained through the Canadian Health Measures Survey (CHMS) and develop pediatric, adult, and geriatric reference intervals for 13 endocrine and special chemistry biomarkers.

Methods: Approximately 12,000 Canadians, 3-79 years of age, participated in the CHMS and provided health information, physical measurements, urine and serum. Thirteen immunoassay biomarkers were measured by the CHMS using the Siemens Immulite 2000, Siemens Advia Centaur XP, Ortho Vitros 5, 1 FS, or DiaSorin Liaison analyzers. CALIPER performed statistical analysis in accordance with CLSI C28-A3 guidelines, using SAS and R software. Subjects were excluded if they were pregnant, using prescription medication, or had a serious medical issue or chronic illness. Scatter and distribution plots were created to visually inspect data and remove extreme outliers. Suspected gender and age partitions were then confirmed by the Harris and Boyd method, the normality of each partition was assessed using Q-Q plots and then the data was transformed using the Box-Cox method. Outliers were removed by the Tukey test or adjusted Tukey test for partitions that were normally distributed or skewed, respectively. The nonparametric or robust method were used to calculate reference intervals for each partition, depending on if the sample size of the partition was >120 or >40 but < 120, respectively. Lastly, 90% confidence intervals were calculated for the endpoints of each reference interval.

Results: Dynamic changes in concentration were observed across pediatric to geriatric age groups. Age-partitioning in reference intervals was required for all 13 analytes and additional gender partitions were necessitated for apolipoprotein (Apo)-AI, homocysteine, ferritin, and high sensitivity C-reactive protein (hsCRP). Apo-A1 levels were slightly higher in males, but remained relatively constant throughout life, while vitamin B12 steadily decreased throughout childhood and then remained constant. ApoB, homocysteine, ferrition, 25-OH vitamin D, RBC folate, serum folate, HbA1c, and insulin all increased with age. Microalbumin, hsCRP, and PTH all showed variable fluctuations across the age range.

Conclusions: The collaboration between CALIPER and the CHMS has enabled a unique examination of the complex biological changes in 13 immunoassay biomarkers over a wide age range. Our rigorous statistical approach, well-defined exclusion criteria and outlier removal procedure has resulted in a robust dataset to permit detailed examination of the normal fluctuations in biomarker levels occurring in apparently healthy individuals. Our analysis not only provides insight into important biological changes that occur with development and aging, but the reference intervals developed here have important clinical implications for improved diagnostic accuracy and patient care.

A-072

Comparison of an Aldosterone Chemiluminescent Immunoassay to a Radioimmunoassay

J. A. Erickson, J. A. Straseski. *ARUP Institute for Clinical and Experimental Pathology, ARUP Laboratories, Salt Lake City, UT*

Background: Aldosterone is a hormone that regulates electrolyte balance and blood pressure. Measurement is used for diagnosing and differentiating primary aldosteronism from the more prevalent secondary aldosteronism. We evaluated the performance of the LIAISON® Aldosterone CLIA (DiaSorin Inc.) and compared it to the recently discontinued Siemens Coat-A-Count® Aldosterone RIA (Siemens Healthcare Diagnostics, Inc.).

Methods: Specimens included deidentified residual serum, urine and heparinized plasma specimens submitted for routine testing, banked adult and pediatric sera and fresh 24-hour urine. Samples were measured according to each manufacturer's protocol. Performance characteristics evaluated included method correlation, analytical sensitivity, linearity, precision and room temperature stability. Serum and urine reference intervals were also verified.

Results: For serum, Deming regression of the CLIA versus the RIA was y=0.85x+5.4, Spearman r=0.959 (n=87). For urine, Deming regression of the CLIA versus the RIA was y=0.60x+113, Spearman r=0.952 (n=40). Utilizing the sample diluent and low aldosterone concentration samples, the CLIA's analytical sensitivity was 0.34 and 1.57 ng/dL for serum and urine, respectively (manufacturer claims: 1.45 and 2.0 ng/dL, respectively). The CLIA's linearity claims for both sample types over the analytical measurement range of 3.0-100 ng/dL were verified using dilutions of highly concentrated aldosterone serum and urine specimens (five pools, four replicates per pool per sample type). Linear regression for serum was y=1.002x+0.253, r^2=1.000; regression for urine was y=1.008x+0.154, r^2=1.000. Utilizing two serum and two urine pools, precision was assessed over 5 days, 1 run per day, 4 replicates per run (20 total observations per concentration). For serum, repeatability CVs were 3.4 and 2.2%, and within-laboratory CVs were 5.7 and 4.4% for Levels I (14.1 ng/dL) and II (36.1 ng/dL), respectively. For urine, repeatability CVs were 2.1 and 3.3%, and within-laboratory CVs were 5.1 and 5.3% for Levels I (15.5 ng/dL) and II (62.5 ng/dL), respectively. Aldosterone was stable at room temperature for a minimum of 8 hours in serum (CVs≤4.0%) and 4 hours in unpreserved urine (CVs≤3.2%). Using banked sera, age-partitioned serum aldosterone reference intervals previously established using the RIA, were verified for the CLIA. For urine, the manufacturer's suggested reference interval of 1.19-28.1 µg/d was verified utilizing fresh 24-hour urine samples from 24 healthy volunteers. Although heparinized plasma is not listed as an acceptable sample type for the CLIA, a comparison study versus the RIA generated a correlation of y=0.87x+2.7, Spearman r=0.952 (n=14). Additionally, paired serum and heparinized plasma specimens taken during the same blood draw produced a correlation of y=1.00x+0.07, Spearman r=0.981 (n=19) for the plasma versus serum.

Conclusions: Comparisons between the LIAISON Aldosterone CLIA and the discontinued Siemens Coat-A-Count Aldosterone RIA using serum and urine samples had correlation coefficients >0.950. The low slope values may possibly due to calibration differences. Despite this, verification of RIA reference intervals indicated similar clinical classification for both assays. These data, along with other acceptable performance characteristics, suggested the CLIA may be a suitable alternative for the RIA. Additionally, heparinized plasma may be considered an acceptable sample type for the CLIA.

A-073

Analytical Performances and Method Comparison Studies of the Beckman Coulter Access 25(OH) Vitamin D Total Assay

C. Cusserne[1], J. Tehranchi[2], S. Bonjean[1], L. Michaud[1], M. Solari[2], M. Navratil[2], S. Villeneuve[1], E. Romeu[1]. *[1]Beckman Coulter, Marseille, France, [2]Beckman Coulter, Chaska, MN*

Back ground and Objectives:

Vitamin D is a lipid-soluble steroid hormone that is produced in the skin through the action of sunlight or is obtained from dietary sources. The role of vitamin D in maintaining homeostasis of calcium and phosphorus is well established. Chronic severe vitamin D deficiency in infants and children causes bone deformation commonly known as rickets, while in adults, proximal muscle weakness, bone pain and osteomalacia may develop. In this study, we assessed the analytical performance of the new Access 25(OH) Vitamin D Total (Beckman Coulter, Brea, USA) assay for Access 2 and DxI platforms.

Methods:

The Access 25(OH) Vitamin D Total assay is a competitive binding immunoassay that uses a vitamin D analogue conjugated to alkaline phosphatase to compete for binding sites on a monoclonal anti-25(OH) vitamin D antibody attached to paramagnetic microparticles. The assay was evaluated for linearity, imprecision and analytical sensitivity using three different reagent lots on three instruments per platform. Equimolar recognition of 25(OH) vitamin D_2 and D_3, cross-reactivity and interfering substances were tested on one instrument and one reagent pack lot on the Access 2 and UniCel DxI 800 platforms. Method comparison studies were performed on 110 samples ranging from 20.0 to 246.5 nmol/L vs. Reference Measurement Procedure (RMP).

Results:

The Access 25(OH) Vitamin D Total assay was found to be linear across the measuring range of 17.5 to 300 nmol/L with an LoB of 3.75 nmol/L, an LoD of 5.0 nmol/L, and an LoQ of 15.0 nmol/L. Within run imprecision ranged from 3.0% to 4.7% on DxI 800 and 1.5% to 3.8% on Access 2, with a total imprecision of 6.6% to 9.3% on DxI 800 and 6.8% to 7.7% on Access 2.

The assay demonstrated equimolar recognition of 25(OH)D_2 and 25(OH)D_3 (Dose ratio 25(OH)D_2/25(OH)D_3: 98% on UniCel DxI 800 and 102% on Access 2), while maintaining good sensitivity and low cross-reactivity with vitamin D metabolites. The Access 25(OH) Vitamin D Total assay was found to have a good correlation with the Joint Committee for Traceability in Laboratory Medicine (JCTLM) approved isotope dilution mass spectrometry (ID-LC-MS/MS) RMP developed at Ghent University[1]. Linear regression results were: y=0.99x-8.46 (r=0.94) on DxI and y=1.01x-7.17 on Access 2 (r=0.95).

Conclusion:

The Access 25(OH) Vitamin D Total assay demonstrated good analytical performance and precision on the Access 2 and DxI 800 platforms. In addition, the assay has equimolar recognition of vitamin 25(OH)D_2 and 25(OH)D_3 and shows a good agreement with the 25(OH) Vitamin D RMP method developed at Ghent University. These attributes indicate the assay is well suited for the automated routine assessment of 25 (OH) vitamin D statuses in patients.

1) Thienpont L, et al. Standardization of measurements of 25-Hydroxyvitamin D3 and D2. Scand J Clin Lab Invest 2012; 72 (Suppl 243): 41-49.

A-074

Novel Anti-Müllerian Hormone ELISAs: Help Diagnose Polycystic Ovary Syndrome*.

A. Kumar[1], B. Kalra[1], A. S. Patel[1], T. Kumar[1], G. Savjani[1], J. A. Visser[2], A. P. N. Themmen[2], L. C. Torchen[3], A. Dunaif[4]. *[1]Ansh Labs, Webster, TX, [2]Dept of Internal Medicine, Erasmus MC, Rotterdam, Netherlands, [3]Division of Endocrinology, Ann & Robert H Lurie Children's Hospital of Chicago, Feinberg School of Medicine, Chicago, IL, [4]Division of Endocrinology, Metabolism and Molecular Medicine, Northwestern University, Chicago, IL*

Objective:

The aim of this study was to measure circulating anti-Müllerian hormone (AMH) levels in women with polycystic ovary syndrome (PCOS) and normal controls using well-characterized AMH ELISAs.

Relevance:

There is a growing interest in the role and measurement of AMH in PCOS. It is a syndrome characterized by hyperandrogenism, ovulatory dysfunction and polycystic ovary morphology. Since the assessment of ovarian morphology requires ultrasonography, there has been considerable interest in identifying biochemical proxies for PCOS-associated changes in folliculogenesis. AMH may be such a proxy.

Within the ovary, highest levels of AMH are expressed by the granulosa cells of small antral follicles <4 mm in diameter. A high circulating AMH concentration identifies women with an unusually high number of small antral follicles. Classically, these will be women with polycystic ovaries. Accordingly, it may be possible to use AMH as a diagnostic tool to differentiate PCOS from its age-matched healthy subjects. Current commercial AMH immunoassays are designed to measure pro-mature AMH complex and may not detect the cleaved pro-region fragment if present in circulation. The development of new immunological methods to measure AMH isoforms in circulation is needed to better understand the role of AMH in PCOS.

Methods:

Two independent ELISA methods (24/32 & 10/24) based on antibody pairing against linear epitopes in the mid-region capture (358-369aa) and mature-region detection (491-502), and pro-region capture (36-47) and mid-region detection (358-369) have

been developed to measure circulating levels of AMH. Serum from 368 PCOS and 192 aged matched control subjects were studied and the diagnostic accuracy was calculated dividing the sum of true positives and true negatives by the total number of subjects.

Results:

The limit of detection of 24/32 and 10/24 ELISAs were 1.0 and 0.5 pg/mL, respectively. Total imprecision measured on two controls (70.4 pg/mL, 221.4 pg/mL) using 24/32 and 10/24 AMH ELISAs over 22 runs were 6.6%, 6.8% and 4.1%, 5.0%, respectively. Linearity of dilution plot (multiple dilutions of 5 samples) resulted in a slope of 1.0 and a p value of <0.0001 in both ELISAs. The median AMH levels for the 24/32 ELISA and 10/24 ELISAs showed significant difference between the control and the PCOS subjects (10.14 vs 2.71 ng/ml, and 6.05 vs 1.78 ng/mL, respectively). ROC analysis for each ELISA was used to establish the cut-offs for diagnosing PCOS subjects (characterized by NIH criteria). The sensitivity, specificity and diagnostic accuracy of 0.84, 0.83, 83.6 at a cut-off of 5.0 ng/mL and 0.85, 0.83, 84.3 at a cut-off of 3.0 ng/mL were observed for 24/32 and 10/24 ELISA, respectively. Higher prevalence of PCOS was observed in sisters of PCOS subjects (43 out of 113 subjects) using the ELISAs.

Conclusion:

Highly sensitive, specific and precise AMH ELISAs have been developed to measure circulating forms of AMH in PCOS subjects. AMH levels in PCOS subjects were highly elevated and were significantly different than the control group. The diagnostic accuracy of 85% was obtained by the novel ELISAs where subjects were characterized by NIH criteria.

* Research Use Only.

A-075

Use of Micro-Liquid Chromatography/Tandem Mass Spectrometry Method to Assess Diurnal Effects on DHEA

L. S. Masika, B. R. Stolze, E. A. Elliott, J. Gu, B. S. Abel, M. C. Skarulis, S. J. Soldin. *NIH, Rockville, MD*

Background: The steroids cortisol, testosterone, 11-deoxycortisol, corticosterone, cortisone, androstenedione, and 17-α-hydroxyprogesterone have all previously been shown to have diurnal variation when measured using micro-liquid chromatography/tandem mass spectrometry (LC/MS-MS) with significantly higher circulating concentrations in the morning (1). Our aim was to assess if diurnal variation could also be observed in the steroid DHEA when measured by LC/MS-MS.

Methods: We measured DHEA using LC/MS-MS in plasma samples from 19 healthy adults drawn at midnight and 0800 the following morning. We used an Agilent 6490 triple-quadrupole LC/MS-MS equipped with an Agilent atmospheric pressure photo ionization (APPI) source and Agilent 1200 HPLC system. 100µL of human plasma was mixed with 150µL of acetonitrile containing deuterated internal standard and vortexed for 30 seconds, then centrifuged for 10 minutes at 13,000 RPM. 150µL of the supernatant was diluted with 250µL of HPLC grade water and vortexed for 10 seconds. Then 300µL of sample was injected into the LC where both DHEA and internal standard undergo an on-line extraction, binary gradient separation and elution. An Agilent Poroshell 120 SB-C8 column was used for chromatographic separation. Quantification by multiple reaction-monitoring (MRM) analysis was performed in the positive mode. The transition selected was: mass-to-charge (m/z) 271.3 to 253.2. Nitrogen served as both the source and collision gas. Circulating DHEA concentrations at midnight and 0800 were compared using paired-sample Student *t*-tests.

Results: Our findings demonstrated a statistically significant difference between DHEA circulating concentrations measured at midnight and 0800 ($p<0.0001$). Mean DHEA increased 60% from 230.7ng/dL at midnight to 566.4ng/dL at 0800.

Conclusion: We demonstrated significant diurnal variation in DHEA concentrations when measured by LC/MS-MS. Time-specific reference values must be generated in order to understand the clinical relevance of DHEA measurements by LC/MS-MS.

References: Stolze et al. *Clinical Chemistry*, 2015; 61(3)

A-076

LC-MS/MS Study of 25-OH Vitamin D2 and D3 with PerkinElmer Vitamin D kit Using both Derivatized and Non-derivatized Methods

H. Qiao[1], J. Ye[1], E. Majdi[1], J. Cournoyer[2]. *[1]IONICS Mass Spectrometry Group Inc., Bolton, ON, Canada, [2]PerkinElmer Inc., Waltham, MA*

Background:

25-OHase enzyme converts Vitamins D2 and D3 to 25-OH Vitamin D in the liver. Quantification of these metabolites is widely used as a means of assessing vitamin D deficiency status because of their clinical significance in a variety of disorders, which lead to alterations in the concentrations of circulating hormones. This work presents a fast, reliable, and accurate LC-MS/MS method on an IONICS 3Q 120 triple quadrupole mass spectrometer for studying 25-OH Vitamin D2 and D3 for research purposes with PerkinElmer Vitamin D kit using both derivatized and non-derivatized methods.

Methods:

The PerkinElmer Vitamin D kit is intended for quantitative determination of 25-OH Vitamin D2 and D3 in human serum and plasma samples. This kit uses a combined solvent extraction and protein precipitation method. It can be used in two alternative ways; non-derivatized, or derivatized. The calibrators are isotope labeled as 2H6-25-OH Vitamin D2 and 2H6-25-OH Vitamin D3 with six levels ranging from 5.4-164.5 ng/mL for 2H6-25-OH Vitamin D2 and 4.6-139 ng/mL for 2H6-25-OH Vitamin D3. Three levels of QC standards were provided, 10, 42.3, and 87ng/mL for 2H6-25-OH Vitamin D2, and 8.7, 35.8 and 73.6ng/mL for 2H6-25-OH Vitamin D3. The IONICS 3Q 120 mass spectrometer was equipped with a heated coaxial flow ion source and "Hot Source-Induce Desolvation" interface for the best ionization and sampling efficiencies. Electrospray ionization was used for this analysis. A Shimadzu Prominence XR UFLC system was used. The column was from Kinetex (C18, 100x2.1mm, 1.7um). The injection volume was 10 µL. A gradient method was created with a flow rate of 0.3mL/min and a total LC cycle time of 4.5 minutes.

Results:

In a 4.5-minute LC run, good chromatogram peak shapes were obtained for both 2H6-25-OH Vitamin D2 and D3. No carryover was detected in a blank injection immediately following the upper level calibration sample. The calibration curves showed good linearity with a coefficient $R2>0.993$ for 2H6-25-OH Vitamin D2 and D3 with non-derivatized and derivatized methods. At the lowest levels for both 2H6-25-OH Vitamin D2 and D3, the accuracies were between 97-102% and CVs were < 10%. For the lowest level in the kit, the S/N ratios of 2H6-25-OH Vitamin D2 were about 70 and 110 for non-derivatized and derivatized, respectively; the S/N ratios of 2H6-25-OH Vitamin D3 were about 40 and 92 for non-derivatized and derivatized , respectively. For 2H6-25-OH Vitamin D2 and D3 in the QC samples with both non-derivatized and derivatized analysis, the accuracies were between 93-109% and the CVs were < 9%.

Conclusion:

A rapid, accurate, and reproducible LC-MS/MS research method was developed on IONICS 3Q 120 mass spectrometer for evaluating the Perkin Elmer Vitamin D kit. The S/N results at the lowest levels indicate that the expected LLOQs for 2H6-25-OH Vitamin D2 and D3 would be at least 4 times lower(~1ng/mL) for non-derivatized and about 10 times lower(~0.5ng/mL) for derivatized methods. Therefore, this LC-MS/MS method with IONICS 3Q 120 mass spectrometer is capable to provide high enough sensitivity, accuracy and reproducibility for quantifying 25-OH Vitamin D2 and D3 with PerkinElmer Vitamin D kit.

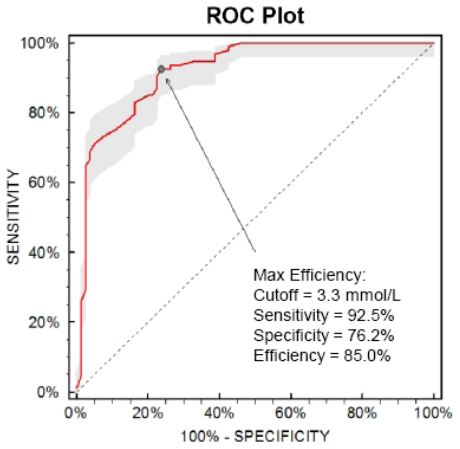
A-077

Determining an Effective Point-of-Care β-Hydroxybutyrate Concentration to Initiate Earlier Treatment of Pediatric Diabetic Ketoacidosis

A. M. Ferguson, J. A. Bartlett, M. Barnes, S. DeLurgio, A. Turpin, U. Garg, J. Michael, M. Clements. *Children's Mercy Hospitals and Clinics, Kansas Ctiy, MO*

Background: Diabetic ketoacidosis (DKA) occurs when ketones accumulate in the blood due to low insulin concentrations. DKA is life-threatening and the leading cause of hospitalization and morbidity in Type 1 diabetic patients. Patients presenting to the ED with suspected DKA need quick assessments and treatments. Our current practice uses a CO_2 value to determine if a DKA Clinical Practice Guideline (CPG) is to be activated. A $CO_2 \geq 16$ mmol/L suggests that DKA is unlikely. However, a $CO_2 < 16$ mmol/L indicates that the patient is acidotic and the DKA CPG is activated. As the

laboratory turnaround time for CO_2 values can be 45 minutes, we wanted to determine if a point-of-care (POC) βHB value could be used to initiate DKA treatment earlier. **Objective:** Determine a best βHB concentration for beginning earlier treatment of patients with suspected DKA. **Method:** A retrospective chart review was done on patients with POC βHB and laboratory CO_2 values within one hour of each other, prior to the initiation of treatment. 173 patients met these criteria. ROC analysis was performed for βHB with CO_2 concentrations used as the gold standard for the diagnosis of DKA. CO_2 values < 16 mmol/L were considered positive and values ≥ 16 mmol/L were considered negative. **Results:** ROC analysis indicated that a βHB value of 3.3 mmol/L predicts DKA with 92.5% sensitivity and 76.2% specificity. The ROC-AUC was 0.922 with an efficiency of 85%. The mean βHB value for the negative cases was 2.07 mmol/L (SD 1.68) and for the positive cases was 5.32 mmol/L (SD 1.49). βHB ranges for negative and positive cases were 0.1-8.0 mmol/L and 1.8-8.7 mmol/L, respectively. **Conclusion:** Measuring blood ketones at POC and using a βHB cutoff of 3.3 mmol/L to initiate a DKA CPG provides treatment sooner without adverse effects or significant cost increases.



A-078

Evaluation of the Cisbio Bioassays Aldosterone (ALDO-ELISA) and Active Renin (RENIN-ELISA) Assays for Determination of the Aldosterone-Renin Ratio for Screening in Primary Hyperaldosteronism

J. Cheung[1], C. Mombrial[2], P. M. Yip[1]. *[1]University Health Network, Toronto, ON, Canada, [2]Cisbio Bioassays, Codolet, France*

Background: The screening of suspected primary hyperaldosteronism (PA) is assessed widely by determination of the aldosterone-renin ratio (ARR). New ELISA methods for serum/plasma aldosterone and plasma renin concentration have become available to replace traditional radioimmunoassays (RIA). This study evaluates two new commercial assays from Cisbio Bioassays for aldosterone and active renin against RIA in a clinical setting, and proposes an ARR cut-off for the screening of PA.

Methods: The study examined 120 patients including 61 normotensive individuals, 39 with essential hypertension, and 20 with a diagnosis of PA who were tested at University Health Network, Toronto, Canada, between May 2014 - February 2015. The Cisbio Bioassays ALDO-ELISA and RENIN-ELISA kits use a 96-well plate format which employs colorimetric detection. For method comparison studies, the predicate RIA methods were the Siemens Coat-A-Count® Aldosterone Assay and Cisbio Bioassays Renin III Generation assay. Paired serum samples and EDTA plasma samples were used for aldosterone and active renin measurements, respectively. Clinical correlation was performed by chart review and an ARR cut-off was determined from Receiver-Operator Characteristic (ROC) curve analysis.

Results: Comparison of serum aldosterone and plasma renin concentrations for the Cisbio Bioassays ELISA methods versus RIA methods showed good agreement with correlation coefficients (r) of 0.918 and 0.976, respectively. For screening of PA, an ARR threshold of 91.8 (pmol/mIU) with a sensitivity of 85% (95% CI: 64.0-94.8) and specificity of 98% (95% CI: 93.0-99.5) was obtained from ROC curve analysis. Using the Cisbio Bioassays ELISA methods, the ability of the ARR to detect PA was very good with an area under the curve (AUC) of 0.963 (95% CI: 0.92-1.01). The positive and negative predictive values at the threshold were 89.5% and 97.0%, respectively.

Conclusion: The Cisbio Bioassays ALDO-ELISA and RENIN-ELISA assays gave comparable performance to the predicate RIA assays for the quantitation of aldosterone and active renin, respectively. Preliminary results suggest that screening for PA can be achieved at an ARR cut-off near 92 (pmol/mIU) using the new ELISA methods.

A-079

Human Serum Leptin Assay on A Multiplex Immunoassay Platform

L. Nguyen, S. Li, B. Yue, W. Li. *NMS Labs, Willow Groove, PA*

***Background*:** Leptin is synthesized in adipose tissue and binds to specific receptors in the hypothalamus to control appetite and energy intake. It has been proposed to be an essential signaling factor to regulate body weight homeostasis and energy balance. Increased leptin concentration suppresses appetite and elevates thermogenesis. Leptin deficiency leads to hypopituitarism and massive obesity.

Objective: The goal of the study was to improve and validate a sensitive and reliable method to accurately quantify leptin concentration in human serum using a Bio-Plex Pro Human Diabetes Assays Kit (Bio-Rad) on a Bio-Plex 200 system (Bio-Rad).

***Methods*:** The assay employs a similar quantitative ELISA format on magnetic beads. Capture antibody is covalently bound to the beads and reacts with leptin in serum. After washing to remove unbound proteins, a biotinylated detection antibody is added to create a sandwich complex. The final detection complex is formed when streptavidin-phycoerythrin (SA-PE) conjugate is added. Laser excites phycoerythrin to generate a reporter fluorescence signal. The concentration of leptin bound to each bead is proportional to the median fluorescence intensity of reporter signal. The leptin assay is performed according to the instructions supplied by the manufacturer with some modifications. Serum (50 µL) is first diluted with standard diluent 1+3 and then diluted with sample diluent 1+9 before being aliquoted in duplicate onto a 96-well microplate. The calibrator is first serially diluted with standard diluent and then each level is diluted with sample diluent 1+9 before being aliquoted onto the microplate in duplicate. Data analysis is performed with Bio-Plex Manager software version 6.1.

***Results*:** Although the Bio-Plex 200 system has a signal output (fluorescence intensity) up to ~25,000 for the leptin assay, the linear signal output is around 10, 000, due to signal saturation. Thus, a linear calibration curve is opted instead of a five-parametric sigmoidal dose-response curve fitting recommended by the manufacturer. The linear calibration greatly improves accuracy and precision with a reasonable linear dynamic range and it is necessary to dilute samples of high concentration level.

The within-run coefficients of variance (CV) are < 6.4% for three levels of quality control samples while between-run CVs are <8.9%. Compared to a quantitative chemiluminescent immunoassay (CIA), the correlation (Excel, simple linear regression) is as follows: $Y = 1.876 * X_{CIA} + 2.7656$, r =0.9068, n=46. The correction is $Y = 2.495 * X_{CIA} + 0.2209$, r =0.9324, n=35, leptin by CIA <10.5 ng/mL, with removal of the high concentration levels. Mean recovery using metrological traceable standard solution (Sigma, L4146, leptin human, >99% by SDS-PAGE, recombinant, expressed in E. *Coli*, lyophilized powder) was 98.5%.

***Conclusions*:** The leptin assay on the Bio-Plex platform (multiplex immunoassay) is successfully validated and is suitable for clinical use in reference laboratory settings. Caution must be exercised when comparing absolute concentration levels from different manufacturers and platforms. It is also necessary to note the assay performance dependence on assay platform, sample type and concentration levels when clinically interpreting the results. Reference intervals for leptin were established.

A-080

Serum 5α-Dihydrotestosterone Measurement by 2D-LC-MS/MS

B. Yue, S. Li, D. Anderson, W. Li. *NMS Labs, Willow Groove, PA*

Backrgounds: In humans, circulating androgen 5α-dihydrotestosterone (DHT) exerts major biological effects on skin and prostate. DHT is a more potent androgen than testosterone (T) and is the primary androgen in the prostate. The DHT concentration also increases with androgen replacement therapy because of T conversion to DHT. The serum DHT concentration and DHT/T ratio are clinically useful for monitoring 5α-reductase deficiency, treatment of benign prostate hyperplasia or prevention of prostate cancer by 5α-reductase inhibitors. The challenges to develop a sensitive, accurate and specific bioanalytical method for DHT include low concentration levels and endogenous T metabolites that may interfere. Radioimmunoassay for DHT measurement requires intensive sample workup and lacks specificity due to cross-reactivity.

Objective: We intend to develop a simple high-throughput assay utilizing two-dimensional liquid chromatography-tandem mass spectrometry with required performance for routine clinical use.

Method: Sample aliquot spiked with internal standard was extracted using a mixture of ethyl acetate and hexane. After vortex mixing, centrifugation, phase separation, complete solvent evaporation, DHT is derivatized with picolinic acid at room temperature and then injected into a 2D-LC-MS/MS system without further purification. An API-5000 triple-quadrupole mass spectrometer (AB Sciex) is coupled to a Shimadzu HPLC system of two sets of binary pumps for 2D-LC-MS/MS. The 1st D-LC uses an Agilent Zorbax 300SB-C3 guard column (12.5 x 2.1 mm) for online extraction and cleanup with 0.5% formic acid in water and methanol as mobile phase while the 2nd D-LC uses a Phenomenex Kinetex C18 (100 x 3.0 mm) for analytical separation using 0.1% formic acid in water and acetonitrile as mobile phase. A six-port switching valve is switched at 1.7 min and 2.2 min to transfer compounds of interest from 1st D to 2nd D in heart-cutting fashion without back flash. The API 5000 is operated in positive electrospray ionization and multiple reaction monitoring (MRM) mode with two MRMs monitored for each analyte or internal standard.

Results: The method was fully validated. The lower limit of quantitation (LLOQ) was validated at 5pg/mL with accuracy >93.8% and total %CV < 8.7%, while the upper limit of quantitation (ULOQ) was validated at 2500pg/mL. Within-run CVs were < 3.0% for three levels of QC samples while between-run CVs were <2.9% and a total CVs <5.6%. The extraction recovery was ~96.2% with matrix effect at ~71.3% and process efficiency of 68.6%. The correlations compared with a reference method (EP Evaluator, Deming Regression, 99% confidence interval to exclude outliers) are as follows: Y = 1.11 * X Reference method + 9.402, r =0.9983, n=37/40, SE =14.053.

Conclusion: The 2D-LC-MS/MS setup allows extensive clean-up and transfers only a small part of elution profile of the 1st dimension containing targeted analyte to the 2nd dimension for high efficiency separation. A simple and sensitive method to accurately quantify DHT in serum by 2D-LC-MS/MS was developed and validated, with a LLOQ of 5 pg/mL and suitable for routine clinical laboratory use.

A-081

The Use of Clinical Equivalence Measured at Different Allowable Total Error to Compare Enzymatic, Immunoturbidimetric & HPLC Methods for the Determination of HbA1c Levels In Patients With Normal and Abnormal Hemoglobin.

S. Saadeddin[1], S. Sobki[1], W. Al-Tamimi[2], G. Elyamany[1], A. Al-Othaim[2], O. Al-Sehibani[1], A. Al-Abdulaaly[1]. *[1]Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [2]King Fahad National Guard Hospital, Riyadh, Saudi Arabia*

Background: High-performance liquid chromatography (HPLC) is considered the acceptable standard measurement procedure for hemoglobin A1c (HbA1c). The objective of this study was to compare and correlate the analytical performance by measuring clinical equivalence(CE) measured at different allowable total error (TEa) of 3 methods for measurement of HbA1c, including HPLC (Tosoh® G8), immunoturbidimetric (Cobas® 6000) and enzymatic (Architect® c4000) methods in patients with normal and abnormal Hb.

Method: Measurements of HbA1c by the three methods were made in blood from 151 patients with normal and 103 patients with abnormal Hb. Intra and inter assay precision of each method was evaluated with control specimens. Results from the Architect® c4000 and from Cobas® 6000 were compared with those of Tosoh® G8 HPLC method to determine correlation & CE at different TEa.

Results: The average HbA1c levels measured by Architect® 4000, Cobas® 6000 and Tosoh® G8 were 6.86 ± 2.16, 6.75±1.27 and 6.96±1.99% for normal Hb samples and 5.81±1.61, 5.70±1.22 and 5.74±1.37% for abnormal Hb samples, respectively. Ten abnormal Hb samples could not be read by at least one machine (6 by Tosoh® G8, 5 by Architect® 4000 and 8 by Cobas® 6000), therefore were not included in the correlation & CE studies. Comparing Architect® c4000 to Tosoh® G8 reviled r=0.9944, y=1.093x-0.720 and CE at an TEa as low as 7% in the normal Hb samples, and r=0.9710, y=1.197x-0.962 and CE at TEa as low as 13% in the abnormal Hb samples. Comparing Cobas® 6000 to Tosoh® G8 reviled r=0.9932, y=0.865x+0.740 and CE at TEa as low as 9% in the normal Hb samples, and r=0.9716, y=0.887x-0.610 and CE at TEa as low as 15% in the abnormal Hb samples. The difference in lowest TEa to achieve CE was mainly observed with HbA1c <5.7 and >6.4%.

Conclusion: Both Architect® c4000 and Cobas® 6000 showed acceptable data quality & correlation with Tosoh® G8 and achieved CE at fairly low TEa for the measurement of HbA1c in patients with normal and abnormal Hb, with Architect® c4000 achieving CE at lower TEa than Cobas® 6000 in both low and high ranges.

A-082

Zooming In on the Low End: Functional Sensitivity of Automated Testosterone Immunoassays

S. L. La'ulu[1], J. A. Straseski[2]. *[1]ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, [2]University of Utah Health Sciences, Department of Pathology, Salt Lake City, UT*

Background: Measurement of testosterone provides an overall assessment of androgen status and aids in diagnosis of several endocrinopathies in men, women, and children. Testosterone concentrations are commonly determined using commercial immunoassays. However, there are well-documented concerns regarding the reliability of these assays at low testosterone concentrations. This is particularly troublesome for patient populations where low testosterone concentrations are expected, such as women and children. While mass spectrometry methods have proven more accurate, adopting these methods is not feasible for all laboratories. The objective of this study was to assess the functional sensitivity (FS) of 5 automated testosterone immunoassays.

Methods: Residual serum samples were obtained following measurement of testosterone using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Samples with similar results were pooled together to prepare 14 human serum pools (pool concentrations 1.2 - 962.5 ng/dL by LC-MS/MS) which were aliquoted and stored at -70°C until use. All pools were tested for total testosterone using the Abbott ARCHITECT $i2000_{SR}$, Beckman Coulter DxI, Roche MODULAR E170, SIEMENS Centaur and IMMULITE 2000 using 2 reagent lots and at least 2 calibrations. Each pool was assayed once per day, 2 days per week for 6 weeks, totaling 12 replicates per pool. Three pools were excluded from analysis on the E170 because the results were below the analytical measurement range. FS was estimated by fitting a power function to the imprecision data and calculating the testosterone concentration that corresponded to a CV of 20% using Excel. Statistical significance between lots (p-value) was calculated using GraphPad Prism.

Results: FS for the DxI, E170, Centaur, and IMMULITE was 10.4, 22.1, 35.5, and 100.5 ng/dL, respectively. The LoQ for LC-MS/MS was previously determined to be 1.0 ng/dL. For the ARCHITECT assay, all pools tested had CVs below our FS definition of 20%, thus true FS could not be calculated. However, the lowest pool measured on the ARCHITECT had a testosterone concentration of 4.0 ng/dL (1.2 ng/dL by LC-MS/MS) with a CV of 2.6%, which confirmed the manufacturer's limit of quantitation (LoQ) claim of ≤4.3 ng/dL. The E170 was the only other method with a manufacturer's LoQ claim (12.0 ng/dL), although our study was not able to meet that claim. Statistically significant lot-to-lot differences were observed for all methods except the Centaur, which contributed to the final CV used in FS calculations. With few exceptions, the ARCHITECT, DxI, E170 and Centaur over-recovered testosterone with an average % recovery of 131, 115, 107, and 168, respectively; whereas the IMMULITE under-recovered (81%).

Conclusions: Significant differences in FS exist among testosterone methods. Some methods showed acceptable performance while others would benefit from assay improvement at lower testosterone concentrations. Lot-to-lot differences contributed to the variability observed, with some methods being more affected by this variability than others. Importantly, in comparison to LC-MS/MS, all methods had a greater tendency to over-recover testosterone except for the IMMULITE. It is critical to understand the accuracy and precision limitations of commercial immunoassays at low testosterone concentrations. This must be considered when evaluating populations where low testosterone concentrations are expected, such as women and children.

A-083

Performance Evaluation of a Prototype Insulin Assay* on the VITROS® ECi Immunodiagnostic System

H. Groth, A. DiMagno, L. Ernst, T. Jakubowski, L. Sprague, K. Teugeman, T. Tubbs, H. Warren, E. Wolniak-Jonasse. *Ortho Clinical Diagnostics, Rochester, NY*

Background: Insulin concentrations in the blood are indicative of endogenous insulin produced by the pancreas. Insulin measurement is important in the management of people with diabetes mellitus and the treatment of insulin resistance. We have developed a prototype assay using monoclonal antibodies provided by Mercodia AB, for the quantitative measurement of insulin in serum for use on the VITROS® ECi Immunodiagnostic System.

Methods: Precision was evaluated by testing a 5 member panel in triplicate 2 times per day for 5 days. Cross reactivity with proinsulin and c-peptide was assessed up to 1000ng/ml; and bovine and porcine insulin were assessed up to 1000µIU/mL. A total

of 134 samples that spanned the assay range were tested in the prototype assay and an aliquot was sent out for testing on a commercially available automated comparator method. The sample set included random samples, fasting samples, post meal samples collected from in house volunteer participants as well as archived samples purchased from a vendor. Reagent stability was evaluated out to 13 weeks.

Results: The total %CVs ranged from 1.1% to 2.4% for precision panel members ranging in concentration from 8 to 218µIU/mL. At 1000ng/mL, the observed % cross reactivity for proinsulin and c-peptide was 0.08% and 0.14%, respectively. At 1000µIU/mL, the observed % cross reactivity for bovine and porcine insulin was 81% and 107%, respectively. For the method comparison, Deming regression analysis yielded a slope of 1.00, intercept of -0.58 and Pearson Correlation Coefficient of 1.00. The overall mean bias for the prototype method was -1.8% as compared to the commercially available automated comparator method. For the stability study, a calibration curve was run at baseline and 5 stability panel members were predicted off this curve at baseline, 2, 4, 6, 8, 12, and 13 weeks using the same preparation of working strength reagents. The largest observed change in predicted concentration at 13 weeks was -2.1%.

Conclusion: Preliminary performance data demonstrate that the prototype assay has acceptable precision, cross reactivity with proinsulin and c-peptide, stability and excellent correlation with a commercially available method.

*Under development

A-084

LC-MS/MS method for the measurement of free 25-OH vitamin D3

J. Gu, B. Stolze, V. Gounden, Z. Zhao, L. Masika, S. Wyckoff, S. J. Soldin. *National Institutes of Helath, Bethesda, MD*

Background: The measurement of total 25-OH Vitamin D_3 is suboptimal with serum concentrations correlating poorly with PTH. For this reason we wished to explore the possibility of quantifying the free fraction employing ultrafiltration at 37°C and LC-MS/MS.

Methods: An AB SCIEX TRIPLE QUAD 6500 tandem mass spectrometer equipped with Atmospheric Pressure Chemical Ionization (APCI) source and Shimadzu HPLC system was employed to perform the analysis using isotope dilution with deuterium labeled internal standard 25-OH Vitamin D_3-d_6. 600 µL of 20 pg/mL internal standard in MeOH was added to the collection cup of a Sartorius VIVASPIN 2 HY ultrafiltration device (10,000 MW cut-off) in advance. 500 µL of human plasma/serum was pipetted to the VIVASPIN 2 ultrafiltration device for centrifugation at 2200 g and 37°C for about 8.5 minutes, when just 300 µL of sample was filtered through the ultrafiltration device. After centrifugation, ultrafiltrate and internal standard mixture was transferred directly to a glass sample vial and vortexed for 10 seconds. 300 µL aliquot was injected onto an Agilent Poroshell 120 SB-C8 column where both 25-OH Vitamin D_3 and internal standard undergo an on-line extraction, gradient chromatographic separation and elution. Quantitation by multiple reaction-monitoring (MRM) analysis was performed in the positive mode. The transitions selected were: mass-to-charge (m/z) 383.3 →229.2 for 25-OH Vitamin D_3 and 389.3 →211.2 for 25-OH Vitamin D_3-d_6. Nitrogen served as curtain and collision gas. The main working parameters of the mass spectrometer were: collision gas 7, curtain gas 35, ion source gas (GS1) 60, nebulizer current 3, probe temperature 350 °C, entrance potential 10 V, and dwell time 50 msec.

Results: The between-day coefficients of variation (CVs) were below 10% for free 25-OH Vitamin D_3 at all concentration tested. Accuracy ranged between 90% and 110%. Good linearity was also obtained within the concentration range of 1-25 pg/mL for free 25-OH Vitamin D_3 ($r \geq 0.995$). The range of results from 34 healthy volunteers was 1.5 to 17.9 pg/mL. This cohort was supplemented with 8 patients with elevated parathyroid hormone (PTH). The free 25-OH Vitamin D_3 concentration correlates excellently with the concentration of PTH and poorly with the total 25-OH Vitamin D_3 concentration. A poor correlation was observed between total 25-OH Vitamin D_3 and PTH.

Conclusion: We describe the first simple, accurate, and fast isotope dilution tandem mass spectrometry method for the measurement of free 25-OH Vitamin D_3 in human serum/plasma samples employing a high sensitivity tandem mass spectrometer. We can now evaluate the role of free 25-OH Vitamin D_3 in patients with bone and/or a variety of malignant diseases.

A-085

Development and performance evaluation of whole PTH (1-84 PTH) assay on LUMIPULSE® G1200

S. ANDO, Y. ISHI, K. MORIYAMA, M. IMAIZUMI, C. OKAMURA. *FUJIREBIO INC., Hachiouji, Tokyo, Japan*

Introduction: Parathyroid hormone (PTH) consists of 84 amino acids, secreted from parathyroid glands. PTH influences calcium and phosphorous homeostasis directly through bone and kidney. PTH concentration in the blood is measured to diagnose hypercalcemia and hypocalcaemia for renal disorder, and to assess parathyroid function in bone and mineral disorders. In the ordinary physical condition, PTH undergoes cleavage. This results in various C-terminal fragments in the blood. And it has been known PTH (1-84) but not these fragments has the bioactivity. We recently developed and launched a fully automated chemiluminescent enzyme assay: Lumipulse whole PTH assay using anti-(1-4) and anti-(39-84) antibodies. This assay detects PTH (1-84), and shows no cross reactivity with any PTH fragments.

Objective: The aim of this study is to evaluate the analytical performance of this new assay.

Materials and Methods: Lumipulse whole PTH is a one-step sandwich assay which uses two goat polyclonal antibodies. On the LUMIPULSE G1200 system, it uses 50 µL of serum or plasma specimen incubated with an ALP-conjugated N-terminal antibody and magnetic particles coated with C-terminal antibody simultaneously. Almost all specimens used in this study were commercial Heparinized or EDTA plasma spiked with PTH. In the correlation study and the reference range study, specimens from the clinical side and healthy donors were utilized.

Results: The intra- and inter-assay precision were assessed by using three plasma spiked with PTH antigen and buffer based PTH solution. The results were 0.6-2.2% and 1.4-1.7%, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were determined as follows: A zero calibrator was measured in replicate of 20, and its average and SD were determined. LOD was calculated as the average +3SD. LOQ (functional sensitivity) is defined as the low range measured value which is its CV (n=20) of 10%. The results were 0.2 pg/mL and less than 1.6 pg/mL, respectively. Measuring range was determined to show a linear standard curve between 4-5000 pg/mL. The dilution linearity was assessed by serially diluted PTH specimens. The specification was the recovery within 100±10%. It was met up to 1:10 dilution. Cross reactivity was evaluated by adding 50,000 pg/mL of each PTH fragment into a specimen. N-terminal, middle and C-terminal fragments exhibited 0.000~0.057% cross reactivities according to CLSI EP7-A2 calculation. These are negligible level for whole PTH measuring in blood. Correlation study of Lumipulse whole PTH with IRMA whole PTH was evaluated. Using Passing-Bablok regression, it showed a slope of 0.98 (95% CI = 0.96 to 1.01) with a correlation coefficient of 0.99 and an intercept of 0.95. The reference range was calculated using 171 healthy volunteer donors by the non-parametric method with reference to CLSI EP28-A3c. The result was 6.8 (90% CI = 5.6 to 7.6) to 38.2 pg/mL (90% CI = 34.1 to 63.3).

Conclusions: Lumipulse whole PTH is the 3rd generation fully automated assay on LUMIPULSE G1200. And the results described above demonstrate its high analytical performances.

A-086

Hypocretin (orexin A / orexin B) and sleep quality in patients with syphilis or HIV positive

S. Tufik[1], M. C. Feres[2], N. A. Raphael[3], A. Barbosa[4], A. A. Lino de Souza[5], D. Mazzotti[3], C. S. Silva[3], S. G. Oliveira[6], D. Poyares[7]. *[1]Associação Fundo de Incentivo a Pesquisa - AFIP, Sao Paulo, Brazil, [2]Universidade Federal de Sao Paulo- Brazil, Sao Paulo, Brazil, [3]Associação Fundo de Incentivo a Pesquisa - AFIP - Sao Paulo Brazil, Sao Paulo, Brazil, [4]Universidade Federal de Sao Paulo - UNIFESP -, Sao Paulo, Brazil, [5]Universiadade Federal de Sao Paulo - UNIFESP, Sao Paulo, Brazil, [6]Universidade Federal de Sao Paulo - UNIFESP, Sao Paulo, Brazil, [7]Universidade Federal de Sao Paulo- UNIFESP, Sao Paulo, Brazil*

Background:

Hypocretin (Hcrt) or Orexin is a neuropeptide that binds to two major receptors: Hcrt 1 (Orex A) and Hcrt 2 (Orex B). Their neurons are located in the lateral hypothalamus, dorsomedial and perifornical area. The deficit Hart directly affects on the sleep-wake cycle, homeostasis, search natural reward in drug abuse and appetite. Narcolepsy, a sleep disorder whose main symptom excessive sleepiness (ES) and is characterized by low levels Hcrt in the cerebrospinal fluid (CSF). However there are reports in

the literature that the variation in the levels of this also can occur in other infectious diseases such as affecting the CNS (Central Nervous System).

Our objective was to investigate the Hcrt levels in the CSF of patients with active or treated syphilis or HIV positive patients and associate these levels the presence of ES.

Methods:

142 consecutive patients in the hospital of sexually transmitted diseases, aged above 18 years, n = 27 women, n = 22 treated syphilis or active n = 41 with previously diagnosed HIV positive and had CSF collection request (lumbar or cisternal) for tests, were evaluated.

The Epworth questionnaire was used to assess the presence of ES in recent months. The CSF sample was taken to measure the concentrations of Hcrt (1 and 2) by enzyme immunoassay method (EIA) using the reagent: Cusabio Human Orexin A e B (ELISA Kit Catalog Number CSB-E08859h).

Results:

Patients with active syphilis or treated showed significantly lower values Hcrt 1 = 63.5. ± 15.8 ng/mL vs 138.0 ± 46.4 ng/mL(p = 0.043) and Hcrt 2 = 547.5 ± 34.7 ng/mL vs 528.8 ± 55.6 ng/mL, p = 0.125) compared to those without syphilis. For HIV positive patients also had significantly lower levels of Hcrt 1 (55.8 ± 24.3 ng/mL vs 106.6 ± 33.1 ng/ml, p = 0.006) and Hcrt 2 (517.8 ± 145.3 ng/mL vs 488.2 ± 93.4 ng/ml, p = 0.08) when compared to HIV negative ones.

For ES level in patients with active or treated syphilis presented (5.4 ± 3.9 vs 7.7 ± 5.7, p = 0.032) compared to negative patients. And was not seen differences between the degree of ES in HIV positive patients (7.3 ± 4.5 vs 7.8 ± 6.1, p = 0.56) when compared to HIV negative. The Spearman correlation test between Hcrt 1 and the ES scale was r = 0.79, while for Hcrt 2 was r = 0.46. te superior do formulário

Conclusion:

Our results demonstrated that Hcrt 1 or orexin A presented is reduced in patients with syphilis and HIV positive, it seems to be related to the degree of ES, but the ES levels found were not severe, and although significantly different, are within the limits of normality, this way, the relationship between reducing agents Hcrt and ES in patients with CNS infection needs further evaluation.

A-087

Development of a Vitamin D Total Assay* Using LOCI Technology on the Dimension EXL Integrated Chemistry System

M. S. Masulli, J. Li, E. D. Garcia, Z. Teng, M. Drinan, C. Hixson, B. Wessel, M. Sharma, T. Q. Wei. *Siemens Healthcare, Newark, DE*

Background:

The Siemens Dimension® EXL™ Integrated Chemistry System incorporates multiple detection technologies, including LOCI® technology, which enables high-sensitivity immunoassay formats. Siemens is currently developing a Vitamin D Total assay for serum and plasma.

Methods:

The Dimension EXL Vitamin D Total assay (VITD) is a homogeneous competitive chemiluminescent immunoassay based on LOCI technology. The assay measures the total 25(OH)vitamin D concentration [comprising both 25(OH)vitamin D_2 and 25(OH)vitamin D_3] in both serum and plasma. Vitamin D Total LOCI reagents include a releasing reagent, biotinylated monoclonal antibody, and two synthetic bead reagents. Patient sample is incubated with the releasing reagent to release 25(OH)vitamin D molecules from the vitamin D-binding proteins. The reaction mixture is then incubated with biotinylated antibody to form a 25(OH)vitamin D/biotinylated antibody complex. Chemibeads coated with a 25(OH)vitamin D_3 analog and chemiluminescent dye are added to remove the excess free biotinylated antibody. Streptavidin-coated Sensibeads containing a photosensitive dye are added to bind the biotinylated antibody. Aggregates of the Chemibead analog/biotinylated antibody/streptavidin Sensibeads are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from the Sensibeads, which diffuses into the Chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is inversely proportional to the concentration of total 25(OH)vitamin D in the sample.

Results:

The method requires 8 µL of serum or plasma. Time to first result is 32 minutes, with stable calibration for 7 days. Three-day open-well stability and 30-day onboard unopened stability have been achieved. Calibrator values are traceableto the Ghent ID-LC/MS/MS 25(OH)vitamin D reference measurement procedure. The VITD method is linear from 4 to 150 ng/mL. Reproducibility was assessed using the CLSI EP5-A2 protocol with serum samples ranging from 16 to 49 ng/mL. Repeatability CVs ranged from 2.2 to 2.5%. Within-lab CVs ranged from 3.3 to 3.9% respectively. Split sample correlation between this method and LC-MS/MS (VDSCP-certified) produced the following statistics: slope = 1.0115, intercept = −2.00 ng/mL, r = 0.9172, and n = 112 over a concentration range of 7.8-71 ng/mL. Minimal cross-reactivity is observed with 1,25$(OH)_2$vitamin D_2 and D_3 at 500 pg/mL, 3-epi-25$(OH)D_3$ at 100 ng/mL, and vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol) at 1000 ng/mL. This assay is equimolar for 25(OH)vitamin D_2 and D_3.

Conclusion:

The Dimension EXL Vitamin D Total assay demonstrates acceptable precision, accuracy, and turnaround time for total 25(OH) vitamin D measurement on the Dimension EXL system.

*Under development. Not available for sale.

A-088

Prototype of accurate and precise immunoassay for low estradiol concentration determination

E. Romeu[1], P. Melchionno[1], N. Germain[1], H. Fonteneau[1], M. Navratil[2]. *[1]Beckman-Coulter, Marseille, France, [2]Beckman-Coulter, Chaska, MN*

Background:

Estradiol immunoassays are prone to inaccuracy at low estradiol concentrations which is detrimental when addressing the clinical status of children, men, postmenopausal women, and women receiving aromatase inhibitors. The aim of the abstract is to present a prototype immunoassay that is able to accurately and precisely measure estradiol concentrations from 16 pg/mL to 5000 pg/mL.

Methods:

Ninety samples covering the physiological variability (male, female, post-menopausal) and the range of estradiol concentrations (1.2-4368 pg/mL) were assayed on a JCTLM approved reference measurement procedure (ID-GC/MS), Siemens Centaur XP (S), Roche Cobas II (R) and Beckman Coulter Access prototype (B) immunoassays. Correlation was assessed using Spearman rank correlation. Method comparison was assessed using Passing Bablok linear regression and Bland-Altman percentage bias. For all analysis, ID-GC/MS estradiol measurement was used as the reference. Limit of Quantification (LoQ) was assessed according to CLSI EP17-A2 by measuring the total imprecision of 9 samples with a minimum of 5 replicates each day over 5 days on both Roche Cobas (R) and Beckman Coulter Access prototype (B) immunoassays.

Results:

The Spearman rank correlation was high for all methods at 0.967, 0.989 and 0.993, for methods R, S and B, respectively. Over the 90 samples, each method failed to quantitate some of the 17 samples below 10 pg/mL due to lack of sensitivity, with 8, 4 and 3 missed samples for methods R, S and B, respectively. The LoQ was determined at 23 and 16 pg/mL for methods R and B respectively which explains the higher number of missed samples on method R. In addition to random variability, methods R and S exhibit a systematic bias at low estradiol concentrations as highlighted by the intercept of the Passing Bablok linear regression of 13, 11 and 2 pg/mL for methods R, S and B, respectively, the last being not statistically different from 0 pg/mL. Focusing on three representative samples at 8.7, 4.6 and 1.6 pg/mL as determined by the reference method the systematic bias is particularly elevated for method R, the Bland-Altman percentage bias ranging +577% to +1900% on those samples assayed at 58.7, 45.2 and 31.6 pg/mL respectively. For method S, the Bland-Altman percentage bias is still elevated, ranging +284% to +824%, the samples assayed at 33.3, 19.7 and 14.6 pg/mL respectively. For method B, the Bland-Altman percentage bias is acceptable, ranging +15% to -58%, the samples assayed at 10, 1.9 and 0.8 pg/mL respectively.

Conclusion:

The Beckman Coulter Access prototype assay is able to accurately and precisely measure low estradiol concentrations which represents more than 50% of routine clinical measurements in general laboratories. In this study the prototype assay was the only immunoassay that was accurate and precise down to 16 pg/mL while other immunoassays were not.

A-089

Early performance evaluation of a new Intact PTH assay* on the Siemens ADVIA Centaur Systems in comparison to a previous assay generation

S. Ray, P. Sibley, K. Wilson, E. Merebet. *Siemens Heathcare Diagnostics, Tarrytown, NY*

Background: Parathyroid hormone (PTH) plays a major role in the regulation of mineral metabolism and skeletal physiology. The continuous advancement of Acridinium Ester (AE) chemiluminescence technology by Siemens Healthcare Diagnostics led to the development of a novel two-site monoclonal sandwich intact PTH assay**.** The first antibody in the Lite reagent, is a mouse anti-human PTH (N-terminal 14-28 region) antibody 2007 26/032 clone labeled with acridinium ester. The second antibody is a biotinylated mouse anti-human PTH (C terminal 52-59 region) antibody clone that is preformed to streptavidin coated paramagnetic latex particles in the solid phase. This is an evaluation of a new monoclonal ADVIA Centaur® intact PTH assay in comparison to the previous polyclonal assay generation in a dialysis laboratory setting, DaVita dialysis center.

Methods: Two hundred and nineteen (219) samples from fresh EDTA plasma specimens were assayed over four runs, one run per day, at DaVita Labs in Deland, FL. The range of specimens was selected to span the measuring limits of the new Investigational Use Only (IUO) PTH assay*. Data were collected from two assays run in parallel - the IUO assay and the current ADVIA Centaur iPTH assay, and analyzed according to CLSI EP-09 using a procedure for method comparison.

Results: All tested controls recovered within their expected dose ranges in each of the four analytical runs. Linear regression statistics were calculated for both assay for 219 patient samples and analyzed using Deming weighted regression. Two different reagent lots of the IUO assay yielded 95% CI for slopes 1.07 to 1.09 and 0.99 to 1.01, respectively, indicating good agreement with the current ADVIA Centaur iPTH assay. The repeatability CVs for the IUO reagent averages 1.6% across the assay range. Limit of Detection (LoD), measured according to CLSI EP17-A2 is found to be 1.6 pg/mL. In addition, the IUO assay has an increased measuring interval 3,000 pg/mL. No high dose hook effect was present at PTH concentrations up to 100,000 pg/mL.

Conclusion: A method comparison between the two assays shows good agreement, indicating users will benefit from an improved overall performance with no apparent shift in the PTH results.

*Under development. Not available for sale.

A-090

Analytical and Clinical Performance of IMMULITE 2000 TSI Assay*

D. Kiaei, N. Birmingham, S. Chapman-Montgomery, B. Zhang, S. Thompson, J. Lei. *Siemens Healthcare Diagnostics, Tarrytown, NY*

Background: Graves' disease (GD) is an autoimmune disorder and the most common cause of hyperthyroidism. In GD, thyroid stimulating immunoglobulins (TSI) bind to the TSH receptor and mimic TSH stimulation of the thyroid gland. The TSH receptor contains a large extracellular domain that presents epitopes for a variety of autoantibodies, including TSI and thyroid blocking immunoglobulins (TBI). In contrast to TSI, TBI bind to the TSH receptor and inhibit TSH stimulation of thyroid cells, leading to hypothyroidism. The IMMULITE® 2000 TSI assay is designed for the specific, quantitative detection of TSI in serum and plasma. The clinical utility of a TSI assay includes a determination of the autoimmune etiology of thyrotoxicosis, monitoring Graves' patient therapy, prediction of remission or relapse, confirmation of Graves' ophthalmopathy, and prediction of hyperthyroidism in neonates.

Methods: The IMMULITE 2000 TSI assay is an automated chemiluminescent immunoassay with a time to first result of 65 minutes. It employs a pair of recombinant human TSH receptor chimeras in a bridging format. The assay is traceable to the WHO 2nd International Standard for Thyroid Stimulating Antibody, NIBSC Code: 08/204.

Results: The detection limits of the assay were determined in accordance with CLSI EP17-A2 as follows: LoB = 0.03 IU/L; LoD = 0.06 IU/L; LoQ = 0.10 IU/L. The analytical measuring range of the assay is 0.10-40 IU/L. A total of 842 serum samples from apparently healthy males and females were analyzed. The results suggest a nonparametric upper 97.5th percentile of 0.07 IU/L. The assay precision was evaluated according to CLSI EP5-A2. The repeatability %CV varied from 3.5% to 7.0% across the assay range. The IMMULITE 2000 TSI assay was compared to the THYRETAIN TSI Reporter BioAssay using 244 serum samples from GD and other thyroid or autoimmune disease patients with the following results: Positive Agreement: 100% (129/129); Negative Agreement: 92.2% (106/115); Overall Agreement: 96.3% (235/244)**.** Serum samples from 236 treated and untreated GD patients, 138 individuals with other thyroid or autoimmune diseases and 200 apparently healthy individuals were evaluated against clinical diagnosis. The TSI values for the patients with other thyroid or autoimmune diseases had an upper limit of 0.39 IU/L. At 0.55 IU/L cut-off, the clinical sensitivity and specificity were 98.3% (232/236) and 99.7% (338/339), respectively.

Conclusion: The IMMULITE 2000 TSI assay is a sensitive quantitative immunoassay for the specific detection of TSI in the routine diagnosis and assessment of GD patients.

**This product is under development and not yet commercially available.*

A-091

Assessment of the Upper Reference Limit of Estradiol III using the software StatisProTM

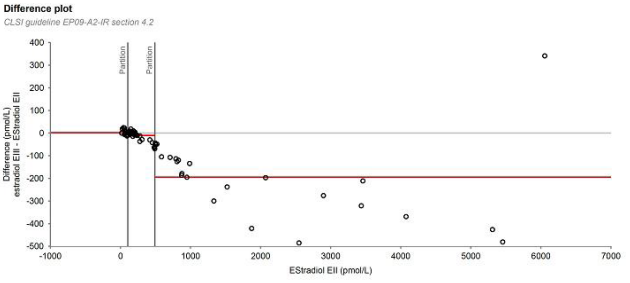
R. M. DORIZZI, P. Maltoni, C. Sgarzani, L. Morotti, E. Bezzi, S. Cotugno. *Core-Lab, The Greater Romagna Area Hub Laboratory,, Pievesestina di Cesena, Italy*

Background. To estimate the reference interval of sexual hormones in females is difficult since they are strongly linked to age and menstrual phase. Recently Roche introduced an assay for Estradiol (EIII) employing two biotinylated monoclonal antibodies (rabbit) that superseded EII assay employing a biotinylated polyclonal antibody (rabbit). Upper Reference Limits (URLs) proposed for EIII in male adults and in females in follicular and luteal phases and menopause are vey similar to those proposed for EII but that proposed for ovulatory phase is significantly lower (30 %). The aim of our study was to assess the capability of StatisPro™ to verify the EIII reference intervals proposed by the manufacturer.

Methods: EII and EIII were measured using Modular E-170 analyzer (Roche, Mannheim, Germany) in 77 serum samples consecutively collected in routine workload. The measurements were carried out in singleton following the EP9 2-IR CLSI standard and the calculations and the graphs were carried out using StatisPro™ (CLSI and Analyse-it, Wayne, USA) and Medcalc (Ostende, Belgium).

Results: The regression equation was EIII= -22-65 + 0.940*EII; Passing and Bablok regression 2.952 + 0.893 EII and the correlation was 0.996 (95% Confidence Interval: 0.994-0.997). The partitioned biases were 2,00 (SD 8.59) at E < 106 pmol/L (n=25); -10.4 (SD 10.61) at E ≥106 and < 490.1 pmol/L (n=27); -194.57 (SD 175.69) at E ≥490.1 pmol/L (n=25) with Sy.x=109.93.

Conclusion: Calculate or, at least, verify the reference intervals of hormones that present large variations dependent on sex, age and menstrual phases is often virtually impossible for laboratorians. StatisPro™ can be really useful since carries out all the calculations and graphs needed for demonstrating the comparability of the results along the concentration span of results yielded by different firms or different reagents. EIII lower URL in ovulatory phase is consistent with the negative bias at high concentration.



A-092

Circulating Bilirubin As A Marker Of Adverse Coronary Heart Disease Risk Profile In First Degree Relatives Of Patients With Type 2 Diabetes Mellitus

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

Introduction & Objectives :

Recent studies have shown that circulating total bilirubin (Tbil), often considered to be a toxic byproduct of haem catabolism, is inversely associated with risk for coronary heart disease (CHD) and diabetic nephropathy. The objective of this study was to examine the associations of TBil with low grade inflammation, circulating adipokines, insulin resistance (IR), metabolic syndrome (MetS) and incident diabetes in first degree relatives (FDR) of diabetic subjects.

Methods

Fasting Tbil, adiponectin, leptin, leptin receptor (sOB-R), insulin, glucose, high-sensitivity CRP (hsCRP), lipid profile were determined in 590 (238M and 352F) FDR. Free leptin index (FLI), insulin sensitivity (%S) and resistance (Homeostasis Model Assessment (HOMA-IR)) were calculated. Patients were categorised by IR, MetS (International Diabetes Federation criteria) and bilirubin quartiles.

Results

Tbil showed significant ($p<0.05$) inverse correlations with BMI ($r = -0.24$), insulin ($r=-0.16$), HOMA-IR ($r=-0.12$), Triglycerides ($r=-0.14$), Apo B ($r = -0.12$), HbA1c ($r=-0.19$) and direct correlations with %S ($r = 0.12$), sOB-R ($r = 0.20$) and FLI ($r=0.24$). Subjects in the first TBil quartile had higher ($p<0.05$) BMI, waist circumference, triglycerides, HbA1c, insulin, HOMAIR, resistin, leptin, FLI, hsCRP and lower HDL-C and adiponectin compared to subjects in the 4th quartile. TBil decreased stepwise with increase in BMI and number of MetS components. The prevalence of MetS from 1st to 4th quartile were 40%, 40%, 13%, and 7%, respectively. The prevalence of IR from 1st to 4th quartile were 30%, 26%, 24%, and 20%, respectively. Binary logistic regression analysis showed odds ratio of the association of TBil with IR, MetS and incident diabetes were 0.88, 0.92 and 0.93 respectively.

Conclusions

TBil in the upper quartile of the "normal" reference interval is associated with healthy metabolic profile and factors that reduce CHD risk . In contrast, TBil in the lowest quartile is associated with increased CHD risk profile, suggesting that TBil may have anti-atherogenic properties. These findings suggest the need for laboratory medicine practitioners to redefine the "normal" reference range for TBil. There is also need for attending physicians to prudently review results of the routinely estimated TBil as low levels could be useful adjunct for the selection of high risk FDR for more aggressive intervention to lower the risk of progression to T2DM or development of CHD.

A-093

Development of a highly sensitive enzyme immunoassay for oxytocin

H. Arakawa[1], S. Haraya[1], K. Karasawa[1], Y. Sano[1], H. Ohkuma[2]. *[1]Showa university, Tokyo, Japan, [2]Eiken Chemical Co. Ltd, Tochigi, 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 this peptide in human social behavior. The levels of oxytocin in plasma have been measured by radioimmunoassays and enzyme immunoassays (EIA), but the many reported methods lack sufficient sensitivity and specificity for oxytocin. We here report the production of a high affinity and high specificity antibody towards oxytocin and its use in a highly sensitive EIA using colorimetric and bioluminescence detection.

Methods:

Antibody: Anti-oxytocin antiserum was obtained by immunizing rabbits with oxytocin-bovine thyrogloblin conjugate prepared by the glutaraldehyde method.

Assay method: Oxytocin sample was added to a second-antibody-coated microtiter plate and reacted overnight at 4 °C. Biotinylated oxytocin was then added for 1 hour at 4 °C, followed by the addition of horse radish peroxidase (HRP)-labeled avidin and incubation for 1 hour at room temperature. The plate was then washed three times with buffer to separate bound/free and the activity of HRP bound to antibody was measured colorimetrically using o-phenylenediamine at 490 nm.

For bioluminescence detection, second-antibody-coated magnetic beads, unlabeled avidin, thermostable biotinylated luciferase, and luciferin as the substrate were used.

Examination of cross-reactivity

The cross-reactivities of three oxytocin-like peptides, [Arg8]-vasopressin (AVP), [Lys8]-vasopressin (LVP), and [Arg8]-vasotocin (VT) with oxytocin were examined by cross-reactivity tests.

Results:

The proposed method is based on the principle of competitive EIA using anti-oxytocin antibody from rabbit and biotinylated oxytocin as the labeled antigen. The labeled antigen comprised biotin chemically bound to oxytocin containing 0 to 5 lysines, providing bridge-link heterology. Six labeled antigens were prepared and used to develop a highly sensitive EIA. Rabbits were immunized with oxytocin bound through the N-terminus to the carrier protein bovine thyroglobulin. The produced antibody and the six biotinylated oxytocins were used in various combinations to probe the sensitivity of the EIA. The sensitivity of the EIA improved as the number of lysine residues increased; consequently, biotinylated oxytocin bridged with 4 or 5 lysines was used thereafter. A standard curve range for oxytocin was 2.5 to 1000 pg / assay. The detection limit of the assay was 2.5 pg and the reproducibility of each point in the standard curve had an average coefficient of variation value ($n = 5$) of 3.18%. The specificity of the assay was tested using three 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 antiserum is very highly specific for oxytocin. In addition, the detection limit for this EIA can be improved to 1.0 pg/assay using a bioluminescence detection method.

Conclusions:

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

A-094

Macroprolactin: Its prevalence and the need to screen in a tertiary care hospital

Y. L. Trull, I. A. Hashim. *University of Texas Southwestern Medical Center, Dallas, TX*

Background: Circulating human prolactin is known to exhibit molecular heterogeneity. The clinical significance of which remains unknown. Macroprolactin, a 150 kDa molecular weight form, may interfere in prolactin assays causing false hyperprolactinemia that may lead to unnecessary additional investigation. However, the practice of screening for the presence of macroprolactin is not widely adopted. We investigated the prevalence of macroprolactin among patients presenting to a large tertiary care teaching hospital and being investigated for fertility disorders.

Methods: Serum samples from patients presenting to Parkland Memorial Hospital with elevated prolactin levels, as determined by laboratory-based immunoassay analyzer (COBAS, Roche Diagnostics, Indianapolis, IN) were retrospectively collected and stored at -20C until further analysis. Stored samples were thawed and mixed with polyethylene glycol (PEG) at 1:1 (v/v) for a final PEG concentration of 12.5% (v/v). Samples were allowed to stand at room temperature for 20 minutes followed by centrifugation at 3000xg for 5 minutes. Prolactin levels prior to and following PEG treatment were measured using ELISA (Calbiotech, Spring Valley, CA). Prolactin percentage recovery following PEG treatment was calculated.

Results: A total of 40 patients' samples were collected during the study period. Prolactin levels as determined by the laboratory-based immunoassay ranged from 37 ng/mL to 244 ng/mL, median 72 ng/mL. This is compared with ELISA-based prolactin levels at 3 ng/mL to 197 ng/mL, median 81 ng/mL. Percentage recovery following PEG treatment for all samples ranged from 30.6% to 127.9 %, median 86%. Samples with percentage recovery less than 40%, greater than 60%, and those between 40 and 60% were considered positive for the presence of macroprolactin, negative for the presence of macroprolactin, and as borderline respectively.

Two samples with prolactin levels of 76 ng/mL and 181 ng/mL were positive for the presence of macroprolactin with a percentage recovery of 30.6% and 35.1% respectively. Four samples at 130 ng/mL, 154 ng/mL, 140 ng/mL, and 147 ng/mL exhibited borderline percentage recovery at 52.4%, 57.2%, 46.8%, and 55.9 % respectively. The remaining study samples had recovery greater than 60% and were thus considered negative for macroprolactin.

Conclusion: Macroprolactin was detected in 6% of samples from patients with prolactin levels greater than 42 ng/mL. Those positive for macroprolactin had prolactin levels elevated at 76 ng/mL and 181 ng/mL.

Although this small study suggests lack of relationship between prolactin levels and the likelihood of macroprolactin presence, further analysis is required to ascertain

the relationship and to investigate the borderline samples. Samples with unexplained elevated prolactin levels should be screened for the presence of macroprolactin.

A-095

Development of immunoassay for detecting liver-type fatty acid binding protein (L-FABP) for LUMIPULSE® G1200

A. Terao, K. Aoyagi, S. Kojima. *FUJIREBIO INC., Tokyo, Japan*

Background: Liver-type fatty acid binding protein, L-FABP, is a 14kDa fatty acid binding protein localized at the proximal renal tubule of human kidney. L-FABPs are known to be excreted through urine during the occurrence of ischemia and/or oxidative stress within the renal tubule prior to kidney tissue damage. Therefore, L-FABP is considered a useful marker in the early diagnosis of diseases that are associated with tubular dysfunction, such as chronic kidney disease (CKD) or diabetic nephropathy. In order to evaluate the progression of such diseases, quantitation of low levels of L-FABP during the early stages is necessary. We have developed the first fully-automated, high sensitive chemiluminescence immunoassay for LUMIPULSE G1200 system for detecting urinary L-FABP, and its performance is evaluated.

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

Results: The detection limit of the assay was 0.03 ng/mL, and the limit of quantitation was 0.16 ng/mL. A 20-day precision study was performed during a 31-day period using two controls and three panel specimens, and the imprecision was ≤ 3.4% total CV. Dilution linearity was evaluated using three test samples, and the recovery rate of up to 100-fold dilution was 95-102% for manual dilution and 99-103% for automated dilution within the calibration range of 0.5 - 400 ng/mL. For spike recovery study, varying amounts of L-FABP were added to urine samples containing low levels of L-FABP to create test samples with concentrations ranging from 70-350 ng/mL. The measured values, when compared to the expected values, ranged from 91-105%. The correlation coefficient and the regression slope of Lumipulse G L-FABP and commercially available enzyme-linked immunosorbent assay kit (CMIC Co., Tokyo, Japan) were 0.96 and 1.07, respectively (N=111). No interference was observed with unconjugated (21.0 mg/dL) or conjugated bilirubin (18.5 mg/dL), hemoglobin (490 mg/dL), NaCl (2 g/dL), glucose (1 g/dL), acetone (100 mg/dL), creatinine (1 g/dL), albumin (1 g/dL), ascorbic acid (500 mg/dL), ethanol (1 g/dL), or riboflavin (10 mg/dL).

Conclusion: The performance of L-FABP assay for LUMIPULSE G1200 was satisfactory, suggesting the possible usage of various types of urine samples including hematuria, albuminuria, and urine with high concentration of ascorbic acid. The evaluation results indicated that L-FABP assay for LUMIPULSE has the ability to precisely quantitate significantly low level samples, and the measurement range of 0.5 - 400 ng/mL may be the widest of existing commercially available assays.

A-096

CLINICAL VALUE OF MEASUREMENT OF THYROTROPIN-RECEPTOR ANTIBODIES (TRAb) IN PATIENTS WITH GRAVES' DISEASE

A. Azizi, P. Sthaneshwar, S. Vethakkan. *University of malaya, Kuala Lumpur, Malaysia*

Background:

Graves' disease (GD) is characterised by stimulating autoantibodies to the TSH-receptor (TRAb). A fully automated immunoassay for the quantitation of TRAb in serum is available on Roche Cobas e411. This study was conducted to evaluate this assay in routine clinical use in the differential diagnosis of thyroid disorders.

Methods:

92 patients who attended a university thyroid clinic were included. Based on TFT results, 48 were classified as hyperthyroid, 18 as hypothyroid and 10 as euthyroid. Five patients who had carcinoma of thyroid and 11 patients who were treated for GD were also included in this study. Of the 48 patients who were hyperthyroid, 34 were diagnosed as GD, four had multi-nodular goitre and 10 were hyperthyroid due to other causes. The sensitivity, specificity, positive and negative predictive values (PPV, NPV) of the TRAb test were calculated using published cut-off values.

Results:

At the cut-off level of 1.6 IU/L (Syme et al, 2011), the sensitivity, specificity, positive and negative predictive values were 88.2%, 100%, 100% and 90.2% respectively. TRAb was positive in three patients who were treated for GD. Using the manufacturer's cut-off value of 1.75 IU/L, the following performance characteristics were found: sensitivity 85.3%, specificity 100%, PPV 100% and NPV 86%.

Conclusion:

Sensitivities, specificities, PPVs and NPVs calculated using the two TRAb cut-off values of 1.6 IU/L and 1.75 IU/L are comparable. TRAb is a useful laboratory test in the differential diagnosis of hyperthyroidism and for the follow-up of patients with GD.

A-098

Verification of CALIPER reference interval for T3 and T4 in Brazilian pediatric population

B. S. Moura, L. G. S. Assunção, M. D. Farace, L. Moutinho. *Lab Rede, Belo Horizonte, Minas Gerais, Brazil*

Background: The Canadian Laboratory Initiative for Pediatric Reference Intervals (CALIPER) established reference intervals (RI) stratified by age and sex for biochemical and hormonal laboratory markers, developing a valuable source of RI for healthy pediatric population. These intervals were determined in the Abbott ARCHITECT System®, providing RI for its users, but useful also for other laboratories, since validated to use for local population and for specific immunoassay platforms. This study objective is to assess, as recommend by CLSI, the applicability of T3 and T4 (free - FT3, FT4, and total - TT3, TT4) CALIPER RI for pediatric Brazilian population, using laboratory database, Lab Rede® - Minas Gerais, Brazil. **Methodology:** Results were collected for children of both sexes, 1-11 months (jan/2013-dec/2014) and 1-18 years (jan-dec/2014). Each analyte was studied with related parameters: TT3 (n=2339) and FT3 (n=565) associated at normal TSH and FT4; TT4 (n=1611) and FT4 (n=1881) associated at normal TSH and TT3. Was used ARCHITECT i2000 platform (Abbott Park, IL, USA), chemiluminescent microparticle immunoassay, serum stored at 2-8°C. The data distribution by age and gender of CALIPER were submitted to EP Evaluator® program to RI verification and statistical analysis. **Results:** The results for groups (central interval of 95%) and their CALIPER RI were approved for FT4 (2 groups) and partially approved for FT3, disapprovingly 1 group on 5. For TT3 and TT4 most of CALIPER RI were rejected, only 1 group was approved on 6, for both. Because insufficient results the verification of FT3 and TT4 in children less than 1 year was disregarded.

Conclusions: It is a challenge to obtain RI for the pediatric population, so the use of database sampling constitutes a viable option for checking the ranges proposed at scientific literature. The CALIPER RI are applicable to the studied population for FT4 and most groups of FT3.

T3 and T4 CALIPER RI verification in Brazilian pediatric population									
TT3 ng/dL Ages (n)	CALIPER RI	RI Verification	Outside	CI 95%	TT4 mcg/dL Ages (n)	CALIPER RI	RI Verification	Outside	CI 95%
1-11 months (44)	84.64-234.38	passes	2,3%	122.25-236.88	1-8 years (351)	6.16-10.32	no passes	14.2%	5.4-10.02
1-11 years (1025)	113.28-189.45	no passes	13.7%	104-204	9-11 years (317)	5.48-9.31	no passes	14.8%	4.8-9.52
12-14 years (506)	97.66-176.43	no passes	10.7%	86.67-182.3	12-13 years female (142)	5.08-8.34	no passes	15.5%	4.66-8.98
15-16 years female (254)	92.45-141.93	no passes	22.8%	78-181.25	12-13 years male (77)	5.01-8.28	no passes	11.7%	4.48-8.51
15-16 years male (95)	93.75-156.25	no passes	15.8%	81.6-154.6	14-18 years female (496)	5.46-12.99	no passes	14.3%	4.48-10.46
17-18 years (415)	89.84-167.97	no passes	18.6%	75.4-193.6	14-18 years male (228)	4.68-8.62	passes	5,7%	4.47-8.33
FT3 pg/mL Ages (n)					FT4 ng/dL Ages (n)				
1-11 years (253)	2.79-4.42	passes	8,7%	2.64-4.56	1-11 months (32)	0.89-1.70	passes	6,3%	*
12-14 years female (59)	2.5-3.95	no passes	15.3%	2.05-4.4	1-18 years (1849)	0.89-1.37	passes	7,8%	0.86-1.40
12-14 years male (56)	2.89-4.33	passes	8,9%	2.6-4.47					
15-18 years female (141)	2.31-3.71	passes	9,2%	2.2-3.75					
15-18 years male (56)	2.25-3.85	passes	8,9%	1.99-3.81					

A-099

Evaluation of Analytical Performance of the Beckman Coulter Total βhCG (5th IS) Immunoassay

K. M. Ness, A. Algeciras-Schimnich. *Mayo Clinic, Rochester, MN*

Background: Clinical uses of human chorionic gonadotropin (hCG) include determination of pregnancy, diagnosis and monitoring of germ cell tumors and gestational trophoblastic diseases, and prenatal screening for Down syndrome when used in combination with other markers. Recently, the first commercially available total βhCG reagent (Beckman Coulter, Inc) calibrated against the WHO 5th International Standard (IS 07/364) was introduced. This new reagent is expected to result in a change in assay analytical performance as well as in a systematic bias when compared to assays standardized against the hCG WHO 3rd International Standard. In this study we evaluated the analytical performance of the **total βhCG (5th IS)** immunoassay.

Methods: Imprecision studies were conducted using Liquichek® Immunoassay and Lyphochek® Fertility quality control (QC) materials (Bio-Rad, Inc). Analyte measurement range (AMR) studies were conducted by testing a lot of hCG calibrators, different than what was used for calibration of the assay, as unknowns. Limit of quantitation (LOQ) studies were conducted using a serum sample with low hCG concentration. Carryover was assessed by running a blank sample after samples containing elevated hCG concentrations. Susceptibility of the assay to heterophile antibodies interference was assessed by evaluating three samples with known interference in the total βhCG (3rd IS) assay. Method comparison studies with the total βhCG (3rd IS) was performed using de-identified serum samples. De-identified samples from 600 patients undergoing 1st or 2nd trimester Down syndrome screening were utilized to derive trimester specific medians.

Results: Intra- and inter-assay imprecision studies produced coefficient of variation (CV) of ≤ 6% (range 3-6%) at concentrations of 4.8, 345 and 16861 IU/L. The AMR of the assay was 0.6 to 1350 IU/L with Passing-Bablok regression fit of y = 1.03x +0.15 (r^2=0.999). Serial dilution (x1000) to expand the AMR produced an average recovery of 100% (range 86-107%). LOQ was determined to be 0.5 ng/mL (CV = 20%). Assay comparison with the total βhCG (3rd IS) assay (n=50, range 0.9-771 IU/L) showed good correlation (r^2=0.992) but a systematic bias with a slope of 1.26 and intercept of 0.26 by Passing-Bablok regression fit. Significant carryover was observed at hCG concentrations ≥120,000 IU/L. The assay was less susceptible to heterophile antibody interference than the total βhCG (3rd IS) assay. Due to the observed bias, adjustment of the coefficients A and B used on the maternal screening median calculation was as follow: 1st trimester, A= 675.292 and B = 0.977; and 2nd trimester, A= 18.758 and B = 41.153.

Conclusion: The analytical performance of the total βhCG (5th IS) was established. The amount of carryover observed might be problematic for laboratories using the assay for tumor marker purposes. Given the expected bias, rebaseline of patients undergoing serial monitoring will be necessary. Laboratories using the assay for maternal screening purposes will need to compute new medians to prevent an adverse impact in the Down syndrome detection rate and false positive rate.

A-100

Prevalence of subclinical hypothyroidism in diabetic patients over 60 years using specific diagnostic criteria for elderly patients, and the impact of treatment on the diagnosis

R. Fontes, O. P. Denardin, J. D. Souza, A. P. M. Almeida, A. C. B. Cerqueira, F. M. C. F. Silva, Y. Schrank, P. B. Araujo, D. M. Gomes. *DASA, Rio de Janeiro, Brazil*

Background: Several studies suggested that hypothyroidism is more frequent in patients with diabetes mellitus (DM), and this would be particularly relevant in elderly. However, there is an adaptation of TSH levels to age, and it is not totally clear how much the medications used for DM treatment or for associated co-morbidities are capable of interfering in TSH levels. The authors tested the occurrence of subclinical hypothyroidism in elderly patients with DM, analyzed the interference of some medication regularly used for DM and for co-morbidities treatment on a group of patients, and if they could change the screening for subclinical hypothyroidism.

Methods:TSH, T4L, and TPOAb were measured prospectively, in 590 patients with DM, older than 60 years (71% women), mean duration of DM of 9.7 years, diagnosed over 2 years before, according to criteria of American Thyroid Association. Groups of patients with treatment without use of medications for co-morbidities (n=378) were patients in only diet regimen, metformin, DPP4 inhibitor, insulin, pioglitazone, and on two or more of these medications. Groups of patients with co-morbidities treatments that could exert potential interference with the TSH level (n=212) were patients in use of propranolol, atenolol, acetil salicylic acid (antiagregant dose), amlodipin, carbamazepine, hydantoin, and of two or more than one drug that could interfere on TSH.

Results: Prevalence of subclinical hypothyroidism in diabetics was 4.1% from 60 to 79 years and 2.6% in ≥ 80 years.There was no significant difference of TSH among groups of treatment for DM (Median TSH- diet only: 1.8 mU/L, metformin:1.7 mU/L, sulfonylurea:1.9 mU/L, DPP4 inhibitor: 1.7 mU/L, insulin: 1.8 mU/L, pioglitazone: 1.7 mU/L, more than one drug: 1.8 mU/L. TSH was significatively higher in patients treated with more than one drug that could potentialy increase its levels, like carbamazepine plus hydantoine or beta blockers plus one of this medications: 2.3 to 2.9 mU/L. TSH was lower in patients in use of amlodipine: 1.2 mU/L. Levels of FT4 were not different in all groups studied

Conclusion: The prevalence of subclinical hypothyroidism was slightly higher in the diabetic group compared to a group of non-diabetic elderly evaluated under similar conditions. Some medications only in associaiton increased TSH. Amlodipine decreased significantly TSH. None of them, as some drugs usually used for the treatment of diabetes mellitus, interfered with the screening of hypothyroidism. This is the first report of the effect of Amlodipine decreasing TSH levels.

A-104

Development of a New High Sensitivity Biochip Based Direct Immunoassay for the-Measurement of Low Levels of Total Testosterone in Serum

T. McConnell, S. Marshall, D. McAleer, M. L. Rodriguez, R. I. McConnell, S. P. FitzGerald. *Randox Laboratories Limited, Crumlin, United Kingdom*

Background:

Testosterone is one of the most commonly measured serum hormones. Accurate and reliable measurement has important clinical implications as this hormone plays significant physiological roles both in men and women. Measurement of the low levels found in male hypogonadism, women and prepubertal subjects is analytically challenging. Immunoassays are used in many clinical laboratories for routine measurement, as they provide rapid and cost effective information regarding circulating testosterone levels; however, it has been reported that immunoassays lack sufficient sensitivity, accuracy and precision when low levels of testosterone are measured. In 2007 the Endocrine Society recommended to carry out the measurements for women and prepubertal subjects only by an extraction liquid chromatography tandem mass spectrometry (LCMS) method due to the inaccuracies of current immunoassays.The current study aimed at developing a new biochip based immunoassay standardized to the industry "Gold Standard" isotope-dilution liquid chromatography tandem mass spectrometry (ID-LCMS-MS) for the detection of low levels of testosterone in serum. This represents an advantageous new analytical tool for endocrinology research and clinical applications.

Methods:

A direct competitive chemiluminescent immunoassay on a biochip platform with the semi-automated Evidence Investigator biochip analyzer was utilized. Assay sensitivity was determined as limit of quantitation (LOQ) in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A; 15 replicates of 4 individual patient samples were tested over 5 independent runs. These samples had predetermined target concentrations from ID-LCMS-MS. Method accuracy was assessed as a measure of bias using the results of LOQ testing. Intra assay precision was determined by the analysis of 20 replicates at 4 clinically relevant concentrations in the assay range. The correlation study was conducted by analyzing 36 serum samples and comparing with the ID-LCMS-MS method.

Results:

The assay was target specific presenting cross-reactivity <1% for estradiol, progesterone, DHEA-S, methyltestosterone, estrone and cortisol.

The LOQ was 0.118 ng/mL within the total allowable error of 25% (set byWestgard). This provided an assay range of 0.118 ng/mL to 15.264ng/mL. The accuracy of this new assay; calculated as a bias of the test result from the LCMS value was + 0.6 %. Intra assay precision expressed as CV (%) for 20 samples at the following concentrations: 0.743, 1.221, 3.393, and 7.036 ng/mL was 6.8%, 5.7%, 6.1%, 6.7%respectively. In the correlation study, linear regression on the resulting data generated r values of 0.9858 for samples with testosterone levels from LOQ 0.118-14.4 ng/mL and 0.915 for female samples in the concentration range 0.118-1.573 ng/mL.

Conclusion:

The results show that the reported new biochip based immunoassay can determine testosterone levels <1.573 ng/mL in serum samples and can be used for the measurement of the low levels found in male hypogonadism, women and prepubertal subjects. This immunoassay is therefore a valuable and reliable new analytical tool for the measurement of low levels of testosterone in serum, which is relevant for endocrinology research and clinical applications.

A-105

Concordance between GH peak and IGF-1 quartiles in 200 short children submitted to GH stimulations test.

Y. Schrank, M. Freire, O. V. P. Denardin, R. Fontes, D. M. V. Gomes, P. B. Mattos, G. Rivelli, O. F. Silva. *DASA - Brazil, Rio de Janeiro, Brazil*

Background:

Although it has been recognized that clinical evaluation is the most valuable aspect of assessment in growth disorders, most clinicians will evaluate children with short stature on the ground of the results of growth hormone stimulating tests (GHST) and insulin-like growth factor-1 (IGF-1) measurement. The assessment of IGF-1 secretion is an important diagnostic tool, as its synthesis is growth hormone (GH) dependent and, on the contrary to GH levels, has relatively stable levels. The diagnosis of GH deficiency seems unquestionable when both GH peak on GHST and IGF-1 concentration are decreased. On the other hand, GH deficiency may be excluded in patients, in whom both GH and IGF-1 secretion are normal. However, in clinical practice, the discrepancies between the results of GHST and IGF-1 secretion are quite frequently observed.

Methods:

The aim of our study was to investigate the relationship of peak stimulated GH and IGF-1 in short stature children. We retrospectively reviewed IGF-1 and GHST levels from 200 consecutive short stature children submitted to simultaneous measurement of IGF-1 and GHST with Clonidine in a Brazilian reference laboratory. Peak GH above 5ng/mL was considered responsive. For comparison among children of different age and sex, IGF-1 concentrations were expressed as IGF-1 quartiles. Serum GH was measured by chemiluminescen immunometric assay and IGF1 by an immunometric chemiluminescence assay. Comparisons of GH peak response between groups were performed using Anova one way

test and correlation between tests was evaluated by Spearman test. $P < 0.05$ was considered statistically significant.

Results:

Our 200 subjects (146 boys and 54 girls) age 10,3 ± 2,9 (mean ± SD) were distributed in accordance to IGF-1 quartiles as follows: quartile 1: 113 (56,5%), quartile 2: 48 (24%), quartile 3: 25 (12,5%) and quartile 4: 14 (7%). Mean GH peak and percentage of responsive GHST did not differ between the quartiles. Albeit we observed a tendency to higher GH peak levels and higher percentage of responsive tests according to crescent IGF-1 quartiles, there was no statistical significance between groups (p respectively: 0,764 and 0,8). Forty seven (24,5%) children did not reach a GH peak above 5ng/mL on GHST. On the other hand, 24 (12%) demonstrated IGF-1 levels below the reference range for sex and age. The concordance between inadequate GH peak and low IGF-1 levels was only 17% and only one third of patients with IGF-1 below reference range had in fact inadequate GH response on GHST.

Conclusions:

Like other studies, we were not able to demonstrate concordance between GH peak at GHST and IGF-1 quartiles. This discrepancy may be explained by several limitations observed in GH stimulation tests and IGF-1 determination. Unfortunately, it is important to consider that we still cannot rely on one single test to confirm or exclude GH deficiency, and subjects with low IGF-1 levels may not be spared of more cumbersome

provocative tests. We remark that clinical judgment should remain the most important tool in the evaluation of short stature and in the interpretation of laboratory tests.

A-107

Is free T3 useful to evaluate thyroid status?

S. Marui, A. L. N. Camilo, D. Waltrick, O. V. P. Denardin. *DASA, Sao Paulo, Brazil*

Background: Thyroid function is always assessed using TSH and free T4 (FT4). However FT4 measurement by immunoassay is affected in special conditions, such as pregnancy, heparin treatment and other unspecific interference. In a small subset of thyrotoxicosis (T3 toxicosis), only T3 is elevated. Usually, free T3 (FT3) measurement is not necessary since total T3 (TT3) suffices. However, FT3 levels may be required to evaluate clinically euthyroid patients who have an altered distribution of binding proteins, especially dysalbuminemia.

Objective: Comparison among total and free thyroid hormones using TSH to define thyroid status.

Methods: We analyzed 1,622 patients' samples from a large database of a private reference clinical laboratory, tested for FT3, FT4, TT3, total T4 (TT4) and TSH all by Chemiluminescence assays (Advia Centaur, Siemens Healthcare). Patients were ranked by TSH into normal range (0.5-5.0 mIU/L), mild elevated (5.0-10 mUI/mL), high TSH (>10 mIU/mL), mild suppressed (0.1-0.5 mIU/mL) and suppressed (<0.1 mIU/mL).

Results: 46.9% were between 18 to 39 years-old, being 1,124 Male: 498 Female. TSH was in the normal range in 57.6% (Table 1). All samples had no differences according to age and gender. We found good correlation between FT3 and TT3 ($r=0.701$, $p<0.001$) and also between FT3 and FT4 ($p=0.702$, $p<0.001$). In the other hand, FT3 and TT3 were not correlated to TSH as FT4 and TT4 were inversely correlated to TSH, as expected ($p=0.018$ and $p<0.001$, respectively). FT3 was correlated only with suppressed TSH ($p<0.001$).

Conclusion: Although some investigators recommend the FT3 assay for monitoring ideal thyroid replacement therapy, in our cohort FT3 levels were not related to TSH ranking, suggesting that its clinical role is not precisely defined. Both FT4 and FT3 determinations must have assay interferences, but only FT4 accurately reports thyroid

status. We conclude that FT3 and TT3 levels are less influenced by normal and borderline TSH ranges.

Table 1: Mean (±SD) of FT3 and FT4 according to TSH ranking				
		FT3(pg/mL)		FT4 (ng/dL)
TSH (mIU/L)		Mean (±SD)		Mean (±SD)
Normal range	935	3.46 (1.76)	810	1.34 (0.49)
Mild elevated	88	4.01 (1.68)	79	1.52 (0.63)
High	39	3.35 (1.59)	38	1.32 (0.66)
Mild suppressed	48	3.52 (1.67)	46	1.42 (0.61)
Suppressed	52	7.39 (8.33)	50	2.39 (1.63)
Total	1,162	3.67 (2.57)	1,023	1.40 (0.65)
Normal values		2.00-4.00		0.7-1.8

A-108

Vitamin D status in Rio de Janeiro: results from vitamin D levels observed in a large reference laboratory

P. B. Araujo, M. C. Freire, Y. Schrank, R. Fontes, D. M. Gomes. *DASA, Rio de Janeiro, Brazil, Rio de Janeiro, Brazil*

Background: Vitamin D is a lipophilic hormone involved in calcium homeostasis and bone metabolism. Although severe 25-hydroxyvitamin D (25[OH]D) deficiency is rare in Brazil, there is accumulating evidence of an increased prevalence of subclinical 25(OH)D deficiency in non-elderly people. The aim of this study was to evaluate 25(OH)D and parathyroid hormone (PTH) status in patients from Rio de Janeiro, Brazil, a city located on the tropics, referred to a large reference laboratory.

Methods: We conducted a retrospective evaluation of consecutive serum measurements of 25(OH)D (chemiluminescent immunoassay, ARCHITECT, Abbott Diagnostics) and PTH (chemiluminescent immunoenzymatic assay, Beckman Coulter), from january to june 2014, obtained from lab LIS database. Patients aged below 12 and above 60 years-old, and patients using glucocorticoids, bisphosphonates, calcium and/or vitamin D replacement were excluded. For comparative analysis, the population was stratified in 3 groups of age: 12-18 (adolescents), 19-30 (young adults) and 31-60 (adults). Levels of vitamin D sufficiency followed recommendations from Endocrine Society as follows: deficiency (<20.0ng/ml), insufficiency (21.1-29.9ng/mL) and adequate (>30.0ng/mL). Statistical significance was determined at $p<0.05$.

Results: A total of 5334 samples were evaluated (78.5% women). Population distributions and analyte results are displayed at Table 1. 25(OH)D inversely correlated with PTH levels ($p<0.001$). Although 25(OH)D result means decreased with increasing age, this difference was not significant ($p=0.243$). On the other hand, the increase in PTH level means significantly correlated with age increase ($p<0.001$).

Discussion: Our survey shows that adequate levels of 25(OH)D are present in 56.1% of the population studied, and deficiency levels are more prevalent among young adults and adults. Although the prevalence of insufficient levels is higher among adolescents, this seems not to be of clinical relevancy, since mean PTH level is lower in this age category. Therefore, a better definition on the optimal level of vitamin D is yet to be determined.

Table 1. Distribution of demographic and laboratory findings.						
Age (n)	**Mean Age (±SD)**	**25(OH)D (±SD)**	**PTH (±SD)**	**25(OH)D status**		
				Deficiency	**Insufficiency**	**Adequate**
12-18 (119)	15.5 ± 1.8	36.32 ± 20.88	30.37 ± 26.32	1.7%	47.1%	51.3%
19-30 (611)	25.75 ± 3.26	35.59 ± 25.23	34.27 ± 37.01	8.2%	33.9%	57.9%
31-60 (4604)	47.44 ± 8.63	34.52 ± 26.30	47.88 ± 90.84	7.6%	36.5%	56.0%
Total (5334)	44.24 ± 11.48	34.69 ± 26.07	45.93 ± 85.55	7.5%	36.4%	56.1%

A-109

Thyroglobulin Measurements to Monitor Reoccurrence in Papillary Thyroid Cancer (PTC) Patients.

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

Introduction: Serum Thyroglobulin (TG) measurements are frequently used as a post operative marker for the follow up of patients with thyroid cancers. Presence of TG antibody (TGAB) interference and analytical issues of various methodologies of TG measurements creates difficulty in the follow up of these patients. Physician's frequently request TG measurements by different methodologies to rule out possible recurrence. This study evaluates TG levels by Chemiluminescent Immunoassay (CIA) and Radioimmunoassay (RIA) methods in both TGAB positive and negative patients with PTC, post thyroidectomy.

Methods: TG and TGAB were measured by both CIA (Siemens Immulite 2000) and RIA (USC Endocrine Labs, Pasadena, CA) from 38 patients (10 TGAB positive and 28 TGAB negative) diagnosed with PTC.

Results: In TGAB positive patients all 15 samples showed measurable TG levels (0.9-10.8 ng/mL) by RIA method, where as only 2 samples showed measurable levels (3.0-3.2 ng/mL) by CIA method. In TGAB negative patients only 9 out of 41 samples showed measurable TG levels (0.2-1.7 ng/mL) by RIA vs 19 out of 38 samples showed levels ranging from 0.3 to 6.4 ng/mL) by CIA. All patients were followed by Ultrasound of Neck measurements and some patients had FNA biopsy to confirm no recurrence.

Thyroglobulin Methodology	**Thyroglobulin Antibody**	
	Positive	**Negative**
CIA	**2*/15**	**19*/38**
RIA	**15*/15**	**9*/41**
*** Measurable TG levels**		

Conclusion: TG levels by both CIA and RIA methods showed measurable levels in both TGAB positive and negative patients post thyroidectomy. In TGAB positive patients RIA method has measurable levels on all (100%) samples; whereas only 2 (13%) samples had measurable levels by CIA method. In TGAB negative patients RIA method has measurable levels in 22% of samples while 50% of samples had measurable levels by CIA. Given the clinical follow up of these patients with no reoccurrence, these small measurable levels have no significant clinical impact for monitoring recurrence.

A-111

HbA1c Quality Control Material

T. J. Hunter, C. Grandjean, D. Reichenbach, Y. Velar. *Bioresource Technology, Inc., Weston, FL*

Background: The Bioresource Technology HbA1c Control is a frozen liquid quality control material used to assess the accuracy and precision of Immunoassay as well as HPLC laboratory test methods used for measurement of HbA1c in patient samples.

Methods: The HbA1c Control is supplied liquid in two levels and consists of a human red blood cell lysate in a preservative mix sourced from normal and diabetic whole blood. Donors are selected based on target ranges of 4.0-6.0% for Level 1 and 10.0-14.0% HbA1c for Level 2.

Results: The Control material was assayed on immunoassay (DCA 2000+) and HPLC (Tosoh G7/G8) platforms. The table compares %HbA1c results of three different lots of the Control by the alternate test methods.

	%HbA1c	
	DCA	**Tosoh**
	2000+	**G7 / G8**
Level 1	5.2	5.3
	5.3	5.4
	5.2	5.4
Level 2	11.9	12.1
	10.5	11.5
	11.5	12.5

Real-time stability studies indicate the HbA1c controls are stable for 90 days closed vial at 2-8°C, 30 days open vial at 2-8°C and 7 days closed vial at 25°C. Accelerated stability studies predict a shelf life of 24 months when stored frozen at -15 to -25°C.

Conclusion: The Bioresource Technology HbA1c Control is a suitable quality control material to monitor the precision and accuracy of immunoassay as well as HPLC HbA1c laboratory test procedures.

A-112

Mild vitamin D deficiency is related to PTH and calcium homoestasis?

S. Marui, F. Fukuoka, F. Prieto, D. C. C. Akiyama, O. V. P. Denardin. *DASA, Sao Paulo, Brazil*

Background: Vitamin D deficiency occurs even in tropical country and is associated with osteoporosis and extra skeletal manifestations, including cardiovascular, immune system and glycemic control. Definition of vitamin D sufficiency is under debate and several Committees suggest that a minimum level of 20 or 30 ng/mL must be necessary related to bone health. Therefore 25(OH)D levels between 30 to 40 ng/mL is the optimal concentration range. Most patients with 25(OH)D levels between 15-to 20 ng/mL have normal serum calcium, phosphorus and PTH. Prolonged and severe vitamin D deficiency reduces calcium and phosphorus intestinal absorption, causing hypocalcemia and secondary hyperparathyroidism.

Objective Analyze serum calcium, phosphorus and PTH levels according to 25(OH)D levels in patients with normal renal function.

Methods: We analyzed 1,383 patients samples from a large database of a private reference clinical laboratory, tested for 25OHD (Chemiluminescence, Architect Abbott), intact PTH (Chemiluminescence, Immulite2000 Siemens), calcium and phosphorus (colorimetric CPC and phosphomolybdate/UV principle, Advia2400 Siemens, respectively) in the period from October till November 2014 (1,010 M: 373F) and grouped as young adults (18 to 35 years-old), adults (36 to 60 ys) and old adults (> 60 ys). Patients were also classified according to 25(OH)D levels in vitamin D insufficiency and deficiency groups, < 30 and < 20 ng/mL, respectively. All patients with primary hyperparathyroidism and kidney failure were excluded.

Results: We found 28% and 43% vitamin D insufficiency and deficiency, respectively in our cohort. PTH levels were inversely related to 25(OH)D status and phosphorus levels and directly related to calcium levels, as expected. Calcium levels correlated to vitamin D insufficiency and deficiency (p=0.010 and p=0.030). Phosphorus levels were not statistically significant related to vitamin D status and PTH levels.

Conclusions: 25(OH)D levels below 30 ng/mL were associated to higher PTH and lower calcium. Therefore vitamin D replacement must be considered, even if phosphorus levels were at normal range, independently of age as bone homeostasis might be affected.

A-113

Characteristics of the New Beckman Coulter Thyroid-Stimulating Hormone Assay (TSH(3rd IS))

M. Szabo, B. Dumke, P. Krohn, C. Lundeen, K. Paulsen, L. Radde, M. Shukarey, W. Dietz. *Beckman Coulter, Chaska, MN*

BACKGROUND: There is a clinical need for TSH assays to exhibit a high degree of sensitivity (LoB, LoD and LoQ) with the ability to accurately and precisely measure TSH concentrations in the hyperthyroid and subclinical hyperthyroid range.

METHODS: Beckman Coulter is redeveloping their TSH assay. The new TSH (3rd IS) assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroid stimulating hormone in human serum and plasma for use on the Access Family of Immunoassay Systems.

RESULTS: This new TSH (3rd IS) assay exhibited improved sensitivity in comparison to other devices currently available with an estimated LoB of <0.001 µIU/mL, an LoD of ≤0.002 µIU/mL, and a 10% total assay CV LoQ concentration of <0.005 µIU/mL. Total assay imprecision for each of four sample pools (concentrations range from 0.02 µIU/mL to 36 µIU/mL) was less than 6%.There was no detectable cross-reactivity to FSH (<0.0003%), LH (<0.00001%), or hCG (<0.00000004%). The assay was robust against common interferences including hemoglobin (500 mg/dL), bilirubin (40 mg/dL), unconjugated bilirubin (40 mg/dL), and triglyceride/intralipid (3,000 mg/dL). The correlation between serum and Li-Heparin plasma samples was y = 1.01x + 0.00. No hook effect was detected up to 1,500 µIU/mL TSH concentration. The correlation between the current Beckman Coulter Access HYPERsensitive hTSH assay and the new TSH (3rd IS) assay was determined by comparing 149 samples with concentrations ranging from 0.004 to 43 µIU/mL. The resulting slope was y = 1.01x + -0.01.

CONCLUSIONS: The new Beckman Coulter TSH (3rd IS) assay is sensitive and sufficiently accurate to precisely measure TSH concentrations in hyperthyroid patient samples down to 0.005 µIU/mL.

A-115

Anti - GAD: Important help in the early diagnosis of type I diabetes

G. H. C. Souza, D. A. G. Zauli, E. Mateo, A. C. S. Ferreira. *Hermes Pardini Institute, Vespasiano, Brazil*

The Type I diabetes is characterized by insufficient production of insulin by the pancreas being called autoimmune destruction, affecting mainly children, adolescents and young adults. The measure of antibodies to glutamic acid decarboxylase (anti - GAD) has been used to estimate the risk of development of Type I diabetes and can be tested previously to the disease establishment, during the period called prediabetes. This study aimed to validate theantibodies to glutamic acid decarboxylase testing by ELISA manufactured by EUROIMMUN AG.

The validation process was performed according to the information contained in the manufacturer's manual (ANTI-GAD IgG - EUROIMMUN AG) through a comparative study between the diagnostic method carried out using Radioimmunoassay (RIA) and ELISA RADIOIMMUNOASSAY -RIE - (Autoantibodies Anti -Glutamic Acid Decarboxylase - RSR Limited). Assays were performed using a total of 60 samples selected from the RIA patients, 30 positive and 30 negative. The samples were compared using the cut-off values: i) values equal to or higher than 10.0 U / mL are considered positive by ELISA; ii) values higher than 1.0 U / mL are considered positive by RIA. The results show that the absolute agreement was only 67%. Twenty pairs were discordant, with a clear prevalence of positive results in the RIA and negative in the ELISA. The kappa index was 0.333 (95% CI: 0.122 to 0.544). In both protocols, a proficiency study with analysis of samples of newly diagnosed patients with type 1 diabetes and serum from healthy controls was conducted. In this study, the ELISA test achieved a better area under the curve than the RIA test, and calibrated ELISA method with the first reference of the World Health Organization. Thus, the ELISA using anti-GAD IgG kit - EUROIMMUN AG test designed to detect antibodies to glutamic acid decarboxylase (anti - GAD) has been validated and approved for routine use in our laboratory.

A-116

Hypothyroidism Prevalence in Term Pregnant Women in Port Harcourt

E. P. Odum, O. Otokunefor. *University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria*

Background

Hypothyroidism in pregnancy is associated with an increased risk of adverse pregnancy and fetal outcomes. Its prevalence varies with different thyroid stimulating hormone (TSH) cut-off values as well as in different regions of the world, with higher prevalence in iodine-deficient regions.

Aim/Objective

To screen for hypothyroidism in pregnant women at term and to determine the association between iodine intake and TSH levels.

Subjects and Methods

The study was conducted in four health care facilities in Port Harcourt, a major city in Nigeria. Consenting pregnant women that came into the facilities for delivery were consecutively enrolled and their serum TSH and free thyroxine (FT4) were analysed using the Vitros ECi/ECiQ immunodiagnostic autoanalyser. Hypothyroidism was defined according to the 2012 joint American Association of Clinical Endocrinologists (AACE) and American Thyroid Association (ATA) recommendation of serum TSH concentration greater than 3.5 mIU/L in the third trimester. Overt hypothyroidism (OH) was defined as serum TSH >3.5 mIU/L with decreased serum FT4 and subclinical hypothyroidism (SCH) was defined as serum TSH > 3.5 mIU/L with normal serum FT4 levels.

Statistical analysis used: Statistical Package for Social Sciences (SPSS) software version 17.0 (SPSS Inc., Chicago, Illinois, USA)

Results

One hundred and seventy-eight pregnant women were included in this study. Mean (SD) TSH and FT4 were 1.9 (1.4) mIU/L and 12.0 (4.8) pmol/L respectively. Twenty-

four (13.5%) women had TSH values greater than 3.5 mIU/L; 20 (11.2%) had SCH and 4 (2.3%) had OH. There was no significant difference between the TSH of those who used iodized salt and those who did not.

Conclusion

The prevalence of hypothyroidism (13.5%) is higher than that reported in some earlier studies. Most of the women with hypothyroidism had SCH. Further studies are required to investigate hypothyroidism prevalence in first and second trimesters and to determine etiology.

Keywords: Hypothyroidism, prevalence, term, pregnant women, Port Harcourt.

A-119

Prevalence of subclinical hypothyroidism in adults without known thyroid disease: An epidemiological study in Chengdu,China

N. Xin, L. Guangquan, W. Weiqing, S. Haolan, L. Tongxing, H. Hengjian, W. Lanlan, L. Guixing. *West China hospital, Sichuan University, Chengdu, China*

Background:

Subclinical hypothyrodism (SH), which has previously been associated with an increased risk for many serious diseases, is a serious threat to human health. However, due to the lack of typical clinical symptoms, SH is rarely to be noticed. As there is a paucity of data on the prevalence of SH in healthy adult population in Sichuan, this four years epidemiological study was conducted in Chengdu to estimate prevalence of SH among healthy adults.

Methods:

All participants answered a questionnaire that included demographic data, reproductive history, smoking history, previous thyroid disease, family history of thyroid disease, etc. and had a blood sample collected to assess levels of thyrotropin, free-thyroxine and free triiodothyronine when enrolled. SH were diagnosed on the basis of laboratory results.

Results:

(1) The prevalence of SH in the overall study population was 15.3% (16894/110484), and in 2011, 2012, 2013 and 2014, the prevalence of SH in Chengdu were 16.5% (365/2209), 15.9% (3017/19034), 15.8% (5362/34017) and 14.8% (8150/55224), respectively. (2)As shown in Table 1, prevalence of SH increased gradually with age strata both in males and females, and SH prevalence in females was higher than that in males ($P<0.05$)

Conclusion:

The prevalence of SH in Chengdu was high, affecting approximately 2 in 10 adults in the study population. Female gender and older age were found to have significant association with SH. Adults in Chengdu, especially female over 40 years old, should regularly check thyroid function and take timely corresponding intervention.

Table 1. Male and Female prevalence of subclinical hypothyroidism in different age strata in Chengdu

Age-strata(Years)	Male(%, n/N)	Female(%, n/N)	Total(%, n/N)
18-39	10.3, 2171/21095	16.6, 3087/18544	13.3, 5258/39639
40-59	10.8, 3899/36033	22.4, 4721/21067	15.1, 8620/57100
60-79	18.0, 1455/8101	27.6, 1124/4068	21.2, 2579/12169
>80	26.1, 325/1245	33.8, 112/331	27.7, 437/1576
Total	11.8, 7850/66474	20.5, 9044/44010	15.3, 16894/110484

A-121

Comparison of the Abbott Architect 2nd Generation Testosterone Assay with a Radioimmunoassay and LC-MS/MS

A. Tecleab, D. Carlow, R. Schofield, G. Ford, M. Fleisher, R. Mendu, M. Pessin, L. Ramanathan. *Memorial Sloan Kettering Cancer Center, New York, NY*

Background: Accurate and precise measurement of testosterone is necessary to diagnose and manage patients with gonadotropin hormone imbalances. Testosterone levels are also measured to monitor disease progression in patients with prostate cancer who undergo antiandrogen therapy. The Abbott Architect 2nd generation testosterone assay (2G-TESTO) is a one step chemiluminescent immunoassay for the determination of total testosterone in human serum or plasma. The assay involves mixing of serum or plasma with diluents and anti-testosterone antibody-coated microparticles followed by incubation, washing and addition of chemiluminescent-labeled testosterone to form chemiluminescence.

Objectives: The objective of this study was to validate the precision, sensitivity, and linearity of Architect 2G-TESTO and to compare this assay with Siemens Coat-A-Count®RIA and LC-MS/MS performed at a national reference lab.

Method: The precision study was conducted using the Architect 2G-TESTO high and low control materials and pooled human serum. Within-day precision was determined by analyzing 10 samples sequentially and between-day precision was obtained over thirty days. The limit of quantitation (LoQ) and limit of blank (LoB) studies were conducted using diluted low QC material (assayed in triplicate) and a water blank (assayed 10 times). The linearity study was performed using the Architect 2G-TESTO calibration material over the range of 95.09 - 907.7 ng/dL. For additional concentrations diluted and undiluted quality control (QC) materials over the range of 0.00 - 1058 ng/dL were used. We compared Architect 2G-TESTO assay with Siemens RIA and LC-MS/MS testosterone assay using patient serum sample collected for routine testosterone determination. The LC-MS/MS method used in the national reference laboratory used online extraction followed by LC-MS/MS (Singh R (2008) Steroid 73:1339-1344). It has a functional sensitivity of 7ng/mL based on the inter-assay precision of 18.8% and an imprecision (CV) below 10.7% at various concentrations spanning the analytical measurement range.

Result: Within-day imprecision (CV) at concentrations of 10.5 and 195.5 ng/dL were 5.8% and 4.2%, respectively. The imprecision for the pooled serum at a concentration of 4.8 ng/dL displayed a CV of 3.6%. Between-day imprecision for low (10.7 ng/dL), medium (73.1 ng/dL) and high (236.3 ng/dL) quality controls were 10.7 ng/dL, 9.2%;73.1 ng/dL, 8.37%;236.3 ng/dl, 8.35%, respectively. The 2SD LoB (95% CI) was 1.435ng/dL and the LoD was verified to be 4.3ng/dL. The assay was linear over the analytical measurement range of 4.3- 1009.4 ng/dL.

The comparison between Siemens RIA and Architect 2G-TESTO (n=40, range 10.00 to 797.0 ng/dL) show spearman correlation coefficient of 0.994 with a slope of 1.39 and intercept of -6.872 by Deming regression analysis. Comparison with the LC-MS/MS method at the reference lab (n=76, range 16.1 to 1249.3 ng/dL) had spearman correlation coefficient of 0.988 with a slope of 1.22 and intercept of 2.96.

Conclusion: Our validation shows that Architect 2G-TESTO has acceptable precision and linearity over the analytical measurement range. Architect 2G-TESTO show excellent sensitivity. The correlation with RIA and LC-MS/MS at low and high testosterone concentrations show that Architect 2G-TESTO were generally higher than the other two assays.

A-122

The analytical performances of two different HbA1c assays (capillary electrophoresis and boronate affinity by HPLC) against ion exchange HPLC.

D. G. R. Canali, M. B. Balestrin, M. W. Seika, Y. S. Medeiros. *Laboratório Médico Santa Luzia, Florianópolis - SC, Brazil*

BACKGROUND: Along the years, HbA1c has been established as an important tool in diagnosis, control of diabetic patients and as a marker of prediabetes risk. Ion exchange HPLC assay has been consolidated as a gold standard to measure HbA1c, but other methodologies like capillary electrophoresis and boronate affinity HPLC have also been widely accepted due to their good performances. In this study, the analytical performance (precision, accuracy, linearity, and detection of hemoglobin variants) in two HbA1c assays (Sebia Capillarys Flex Piercing® -capillary electrophoresis and Trinity Biotech-Premier HB9210® - boronate affinity HPCL) versus Bio-Rad Variant II turbo® (ion exchange HPLC) platform was evaluated.

RESULTS: Samples with results near medical decision points measured in Bio-Rad Variant II Turbo were selected. All studies were evaluated using CLSI guidelines. Within-run (Capillarys Flex Piercing® values: 5.8 and 6.6%; Premier HB9210® values: 6.7 and 7.0%) and between-run (Capillarys Flex Piercing® values:5.8 and 6.7%; Premier HB9210® values: 5.8 and 6.6%) imprecision values (CV%) were less than 1.8 (laboratory allowable error for all range of results = 2.4%). Accuracy values were 94 and 90% for capillarys electrophoresis and boronate affinity HPCL, respectively. Comparison of both methods against Bio-Rad Variant Turbo II demonstrated significant correlation (Capillarys Flex Piercing®: r = 0.99.; slope = 1.0; intercept = 0.11; Premier HB9210®: r = 0.99; slope = 0.94; intercept = 0.3). Hemoglobin variants (HbS, HbC and HbF: 0,3 to 10,6%) as well as samples from patients with beta-thalassemia trait did not interfere in the tested assays as demonstrated by NGSDP (http://www.ngsp.org).

CONCLUSIONS: These results indicate that both assays present good precision and accuracy values besides no interference with the most common hemoglobin variants. Thus, both assays are alternative options for large volume testing HbA1c in clinical laboratories.

A-123

Evaluation of the Premier Hb9210 HbA1c analyser

A. Schindler[1], K. Balasubramaniam[1], E. A. Elliger[2], S. Schwarzer[1], H. G. Wahl[2]. *[1]Medizinisches Labor Wahl, Luedenscheid, Germany, [2]Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics; University Hospital Giessen and Marburg GmbH, Campus Marburg, Germany*

Background:

The American Diabetes Association (ADA) as well as other national Diabetes Organizations now recommend using HbA1c to diagnose diabetes using a National Glycohemoglobin Standardization Program (NGSP)-certified method and a cutoff of HbA1c ≥6.5%. The requirements for a device to be used as an aid in diagnosis of diabetes consequently became more rigorous, demanding very low level of imprecision at HbA1c from 5% to 12%, specifically CVs <2% (NGSP). In addition little to no interference from hemoglobin variants or substances is asked for. Here we report the evaluation of the Premier Hb9210 (Trinity Biotech USA Inc, Jamestown, NY), a boronate affinity high performance liquid chromatography (HPLC) system. The glycation specific binding of boronate affinity detects all of the glycated Hb species present in the sample and results in one glycated and one non-glycated peak in the chromatogram. The area of the glycated peak, correlating directly to the HbA1c, is then converted to a HbA1c value. Sample capacity is 210 in both batch or continuous loading mode with a sample analysis time of 66 seconds. Minimum sample requirements are 10 microliter whole blood (Dilution 1 : 150) or 5 microliter packed red blood cells (Dilution 1 : 300). Column temperature is 55°C and detection is done at413 +/- 2nm (LED).

Methods

Imprecision of the assay was evaluated according to CLSI protocol. Intra-assay imprecision was done with four patients pools and two quality controls, inter-assay imprecision with two quality controls. Whole blood (EDTA) samples (n= 197) from healthy controls, diabetic patients and patients on hemodialysis were analysed for HbA1c values on the Premier Hb9210, Tosoh HLC-723 G7, Bio-Rad D-10, Menarini HA 8160 and Roche Cobas Integra 800. Method comparison was done by Passing/Bablock and Bland/Altman. Interferences were tested for haemoglobin variants AS, AC, AD, AE and elevated F. Possible interferences from acetylated or carbamylated Hb were tested with in vitro acetylated (Acetaldehyde, acetylsalicylic acid) and carbamylated red blood cells (urea, potassium cyanate).

Results

Inter-assay imprecision (n=20) for the Premier Hb9210 ranged from 1.52 % (HbA1c 5.69%) to 1.58 % (HbA1c 11.47%) and from 1.79 % (HbA1c 38.67 mmol/mol) to 1.96 % (HbA1c 101.87 mmol/mol). Intra-assay imprecision (n=10) for the Premier Hb9210 ranged from 0.43 % (HbA1c 5.42%) to 0.86 % (HbA1c 13.22 %) and from 0.71 % (HbA1c 35.70 mmol/mol) to 1.04 % (HbA1c 121.02 mmol/mol). The imprecision data from the other HPLC systems were in the same range as the Premier Hb9210, but somewhat higher, whereas the Roche system showed CVs higher than 2.0 %. Acetylation caused higher HbA1c values for the Tosoh and Bio-Rad system and Carbamylation only for the Bio-Rad system. None of the haemoglobin variants showed significant interferences for the Premier Hb9210. Over all the analyser showed good correlations.

Conclusion

ThePremier Hb9210 performs well with a short analysis time, so far no detected interferences and is very well suitable for routine analysis of HbA1c.

A-124

Prevalence of Thyroid Antibodies and Thyroid Dysfunction in a Healthy South African Population

C. P. Onyenekwu[1], M. Hoffmann[1], F. Smit[2], T. E. Matsha[3], R. T. Erasmus[1]. *[1]Department of Chemical Pathology, Tygerberg Hospital and the National Health Laboratory Services, Stellenbosch University, Cape Town, South Africa, [2]Department of Biomedical Sciences, Cape Peninsula University of Technology and PathCare Laboratories, Mediclinic Vergelegen, Cape Town, South Africa, [3]Department of Biomedical Sciences, Cape Peninsula University of Technology, Cape Town, South Africa*

Background:

Autoimmune thyroid disease is characterised by antibodies to antigenic elements of the thyroid gland such as thyroglobulin (TgAb) and thyroid peroxidase (TPOAb), in the presence of thyroid dysfunction. The subclinical and clinical forms of thyroid disorders have been associated with hyperlipidaemia, neuropsychiatric disorders and cardiovascular diseases. Little is known about prevalence rates of the above in the South African population. The study objectives were to determine the prevalence of thyroid dysfunction and antithyroid antibodies in a cohort of healthy South African adults; and to assess gender, age or ethnic-related differences in the prevalence rates of anti-thyroid antibodies.

Methods:

A total of 627 adults were recruited from the general public. Venous samples were analysed for free T3 (fT3), Free T4 (fT4), thyroid stimulating (TSH), and thyroid autoantibodies (TGAb and TPOAb). Thyroid dysfunction and antibody positivity were assessed using current reference intervals.

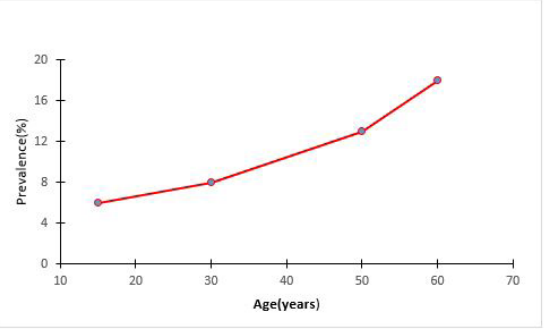
Results:

There were a total of 627 participants aged 18 - 76yrs, 420 (67.0%) of these were females. Participants were of Caucasian (54.0%), Mixed (34%) or African (12%) ethnicity. Subclinical hypothyroidism occurred in 21 (3.3%) adults, while subclinical hyperthyroidism was observed in 4 (0.6%) adults. Six (1.0%) participants were biochemically hyperthyroid while 4 (0.6%) were biochemically hypothyroid. Thyroid antibodies were positive in 55 (9.0%) of study participants. No gender differences in antibody positivity were observed. The highest prevalence of thyroid antibodies were observed in Caucasian subjects (10.0%) followed by subjects of Mixed ancestry (9.0%), with the lowest prevalence (3.0%) seen amongst African subjects. TSH levels ($p<0.01$) and age ($p<0.01$) were significantly higher in seropositive subjects when compared to seronegative individuals.

Conclusion:

Thyroid dysfunction was observed in 35 (5.6%) of participants while thyroid antibodies were present in 55 (9.0%) of participants. Age and ethnic differences were observed in seropositive vs. seronegative participants and a follow-up study is recommended to explore the clinical implications of seropositivity.

Figure 1. Prevalence of Thyroid Antibodies in the various age groups (There is a rise in the prevalence with increasing age.



A-125

Influence of high-normal serum TSH and metabolic syndrome presence in young patients with premature coronary artery disease (age < 45 years)

M. G. CASTELO[1], D. Gonçalves[1], R. Dias Filho[2], A. Pereira[2], G. Coelho[1], T. C. Sousa[1], C. F. Pereira[3], O. P. Denardim[3], T. Bandeira[1], F. Furtado[1]. *[1]DASA, FORTALEZA, Brazil, [2]USP, São Paulo, Brazil, [3]DASA, São Paulo, Brazil*

Background:

The existence of an association between thyrotropin (thyroid stimulating hormone, TSH) levels and cardiovascular risk factor in euthyroid subjects is controversial. We examined the TSH levels and the presence of metabolic syndrome in patients with premature coronary artery (PCAD) disease (age < 45 years).

Methods:

This was a cross-sectional study conducted from November 2010 to January 2015 which included 103 young patients under age 45 years, both sex, with acute myocardial infarction (AMI) diagnosis. We defined metabolic syndrome using the 2007 International Diabetes Federation criteria. The TSH parameter of these patients with PCAD and 267 age and sex matched euthyroid controls without family history were evaluated. In addition, the patients were classified according to the number of affected arteries into 2 groups: single vessel (greater than 50% involvement in

a coronary artery) and multivessels (two or more coronary artery involvement). Comparisons were made between TSH levels of these two groups.

Results: Mean serum TSH was significantly higher in patients with PCAD than controls (TSH 2,6±2,61 *versus* **1,8±0,1 mUI/L;** ***p*****: 0,001). The comparison between single-vessel and multivessel groups showed higher TSH levels in PACD with multivessel affected (TSH 2,3±1,5** *versus* **2,8±1,3 mUI/L;** *p:* **0,01). The prevalence of metabolic syndrome was significantly higher in patients with PCAD than controls (45,65%** *vs* **21,78%;** ***p*****: 0,001).**

Conclusion:

Although observational studies indicate that serum TSH levels in the high normal range are related to cardiovascular risk factor, similar date are limited in young patients with PACD. In conclusion, the prevalence of metabolic syndrome and higher TSH level within the normal range were more frequent in patients with PACD. This pioneering study invites to discussion if TSH levels may be related to more aggressive coronary disease in euthyroid subjects.

A-126

Analysis of TSH, thyroid hormones and reverse T3 in elderly patients and its relation to the Index of Activity Daily Living

R. Fontes, C. A. Pereira, A. S. Santos, D. M. Gomes, P. B. Araujo, M. F. C. Pinheiro, Y. Schrank. *DASA, Rio de Janeiro, Brazil*

Background: It is assumed that elderly subjects could have decreased T3 and increase of reverse T3 (rT3). The Index of Activity Daily Living developed by Katz (IADL Index) assess the functionality of the elderly in six functions called Daily Life Basic Activities - bathing, dressing, feeding, toileting, transferring, and continence. The zero index ranks the independent, and index 6, classifies the totally dependent elderly. The authors assessed the correlations of TSH and thyroid hormones in elderly subjects and tested if TSH and rT3 levels are different in those with different IADL Index.

Methods: We studied 129 patients 65 to 93 years (mean = 80 years), 104 women, who attended a geriatric outpatient clinic. Patients were submitted to a questionnaire that verified the functional status by observing the six daily life basic activities according to Katz index, which are classified in levels 1-6: index 0 indicates the independent individual to perform all activities, and index 6 indicates dependende to perform all of them. TSH, FT4, TT3 and FT3 were measured by quimiluminescence. rT3 was measured by radioimmunoassay.

Results: There was a negative correlation of TSH with fT4 ($r = -0.1736$, $p = 0.0417$), with TT3 ($r = -0.2035$, $p = 0.0167$), and especially with fT3 ($r = -0.2331$, $p = 0.0059$). TSH increased with age, as well rT3, but a positive correlation among them was not quite significant ($r = 0.06687$, $p = 0.0734$). In the same way, correlations among rT3 and fT4 ($r = -0.1143$, $p = 0.2006$), TT3 ($r = -0.08712$, $p = 0.3301$), and fT3 ($r = -0.1109$, $p = 0.2147$) were not significant. Patients were categorized in Katz index 0, 1, and 3. In the segmentation of TSH levels among the groups, there was not significant difference. In respect to rT3, it was significantly higher in the group with index 3 (median rT3 = 0.2900) compared to those with index 0 and index 1 (median rT3= 0.2300 and 0.2100 respectively), $p = 0.0047$.

Conclusion: In this study, the hormone that showed the best correlation with TSH in elderly was fT3. This is in according with previous reports of decreased T3 in elderly subjects without significant decrease of T4. Despite a tendency to increase in rT3, we did not find a significant correlation with TSH.

In relation to IADL rT3 was higher in patients more dependent (Katz index 3), compared with those dependent in only one function ((Katz index 1), and with those completely independent (Katz index 0). Previous studies in literature showed worst physical performance in elderly with higher rT3 despite normal fT4.