Assessing macroprolactin interference in prolactin assays after polyethylene glycol precipitation in two automatized platforms

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Background: Prolactin serum levels over 30 ng/mL in the absence of pregnancy and postpartum breastfeeding are indicators of hyperprolactinaemia which could impact in more complexes and expensive diagnostic protocols. Seric Prolactin are classified in three main forms: monomeric which is the predominant form; dimer, also known as big prolactin; and the high molecular weight form which is usually known as macroprolactin or big-big prolactin (bbPRL). It is known that macroprolactinaemia may correspond to approximately 20 - 25% of cases of hyperprolactinaemia and it is a common disorder in a healthy population. Thus, the investigation of macroprolactinaemia as the main cause of hyperprolactinaemia would avoid clinical investigation of pro lactocinoma and other diseases. The reference test for detecting macroprolactin is gel filtration chromatography, but the test based on polyethylene glycol (PEG) is simpler and cheaper and has been validated in 1999 by Olokoga and Kane. All commercially available prolactin immunoassays have a cross-reactivity level with macroprolactin. The purpose of this study was to validate the PEG precipitation test using Siemens Healthcare Diagnostics ADVIA Centaur System when compared to Abbott Architect.

Methods: 46 patient samples presenting levels over 30 ng/mL previously dosed were re-tested in ADVIA Centaur and Abbott Architect after PEG treatment. Analysis of mean, standard deviation and correlation were calculated. Moreover, a cutoff of 60% was established to determine the presence of bbPRL.

Results: The samples were measured before extraction and the results were: 69.29 ng/mL average in ADVIA Centaur and 94.42 ng/mL in Architect with a standard deviation of 30.12 and 42.10 respectively and R2 of 0.88. 60% was regarded as cutoff for bbPRL screening. When comparing the ADVIA Centaur with Architect, a relative sensitivity of 100% and a Relative Specificity of 83.33% with positive predictive value of 97.56% and negative predictive value of 100% were obtained.

Conclusion: The Prolactin assay varies according to the selected methodology for Macroprolactin detection. Correlation results between compared instruments were satisfactory. Moreover, ADVIA Centaur is capable of dosing the three main forms of seric prolactin. Among 46 samples only one sample showed doubtful result for Architect and positive for ADVIA Centaur. As we can observe macroprolactin is a major interference source and may lead to diagnostic errors and processing errors involving patients with hyperprolactinaemia. Samples present greater dispersion measurements prior to PEG precipitation for Architect results and the difference between the values of pure samples can be related to low interference that ADVIA Centaur system presents, being considered as a positive point once risk in releasing high results decreases. This provides a smaller number of high results which can generate inadequate diagnosis or request a further test to make the diagnosis. In conclusion, we can confirm that the evaluation of bbPRL methods, such as PEG precipitation is still necessary, even in trials that have low reactivity for macroprolactin as the ADVIA Centaur.

Evaluation of Hb A1c bias and precision across eight platforms in the presence of Hb AS and Hb AC

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Introduction: Changes in serial Hb A1c results are due to changes in the clinical condition or to inherent sources of biologic and analytic variation. Results of the 2012 CAP GHZ-B proficiency survey which contained an Hb AS sample, suggest that samples containing an Hb variant may impact assay precision for some methods, and potentially reference change values (RCVs). Objective: To calculate the effect of Hb variants on RCV for Hb A1c by determining the precision of each method. Methods: Seven different NGSP-certified Hb A1c platforms were used to measure precision and bias using patient samples containing Hb AA, Hb AS, or Hb AC. Precision was determined following CLSI EP05-A2, and bias by calculating the percent difference between each test method and the comparative method (NGSP Secondary Reference Lab). RCVs were calculated using the standard formula. Results: Differences in imprecision and bias were observed, and were typically greater in samples containing an Hb variant. RCVs for all methods except two at were ≤0.5% Hb A1c; overall, RCVs were slightly increased in the presence of Hb AS and Hb AC at ≤0.8%. Conclusions: The total analytical error for the majority of assays was significantly greater in samples containing Hb AS or Hb AC, and may indicate a need for including proficiency samples containing the most common Hb variants, especially when assays are used to measure Hb A1c in populations with a high prevalence of Hb variants. In conclusion, while the clinical relevance of increased was beyond the scope of this study, the change in RCV suggests a difference in how serial Hb A1c results may be interpreted.
The difference between the two methodologies is constant, statistically significant. Regarding extreme glycaemia (under 60 mg/dL and greater than 99 mg/dL), all values is -9.624 mg/dL (-10.11 to -9.137) measured by blood glucose strips and enzymatic method. The value assayed by the enzymatic method is -10.87 mg/dL (-11.0 to 10.64) lower than the blood glucose strips.

We observed that in many patients glucose measured at the bedside shows superior plasma glucose with blood glucose strips. Decisions like, correction of blood glucose levels, implementing corrective or alternative treatment, as well as administering another dose of insulin, are based on these values.

Bedside blood glucose control is done, during the lab test, through the measurement of plasma glucose. The given insulin should be able to reduce 50% of fasting glucose or reaching below 100 mg/dL. It is even more important when there is a long time for the release of the operating systems in laboratories of large routine. We observed that the time between January and April 2012, the disparate controls number decreased to 7.8%.

Conclusions: There are statistically significant and consistent difference between the two methods compared. In extreme glycaemia (outside normal range) this difference is even greater. Our data show that if the glucose levels at the bedside (strips) were held on average 10 mg/dL of hypoglycemia target to be achieved in the plasma assay by enzymatic method, we can increase exam security without compromising its reliability and possibly reduce clinical patient discomfort.

Is it possible to make insulin tolerance test (ITT) better?

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Background: The diagnosis of growth hormone (GH) deficiency in children with growth retardation is complex. Because of the pattern of pulsatile secretion of GH, isolated determinations have no value. So, the functional stimulus tests for evaluation of GH secretory reserve are fundamental for the diagnosis. The Insulin Tolerance Test (ITT) is considered to be the gold standard test for diagnosis in children over 2 years of age. This test consists on the administration of intravenous insulin and sampling a multi amstral sequence of glucose and GH, and sometimes, cortisol.

The given insulin should be able to reduce 50% of fasting glucose or reaching below the normal range, promoting the stimulus needed to boost the production and release of GH and cortisol.

Bedside blood glucose control is done, during the lab test, through the measurement of plasma glucose with blood glucose strips. Decisions like, correction of blood glucose as well as administering another dose of insulin, are based on these values.

We observed that in many patients glucose measured at the bedside shows superior values to subsequently assayed by enzymatic method (hexokinase). This study aims to determine the difference between plasma glucose measured by blood glucose strips (bedside) and enzymatic method and propose new targets for bedside blood glucose to increase the security of ITT.

Methods: From March 2013 to January 2014 we conducted 423 ITT. In all these measurements of plasma glucose were performed using blood glucose strips (bedside) and enzymatic method. The blood sampling was done at the same time for both methods. The results were matched and compared statistically.

Results: There is statistically significant difference between the plasma glucose levels measured by blood glucose strips and enzymatic method. The value assayed by the enzymatic method is smaller than the other (bedside). The median difference between all values is -9.624 mg/dL (-10.11 to -9.137)

Regarding extreme glycaemia (under 60 mg/dL and greater than 99 mg/dL), the difference between the two methodologies is constant, statistically significant and even higher. The enzymatic method is -10.87 mg/dL (-11.0 to 10.64) lower than the equivalent sample at the bedside method (strips). At standard deviation (SD) we found a deviation of +/- 21.75 mg/dL.

Conclusion: There are statistically significant and consistent difference between the two methods compared. In extreme glycaemia (outside normal range) this difference is even greater. Our data show that if the glucose levels at the bedside (strips) were held on average 10 mg/dL of hypoglycemia target to be achieved in the plasma assay by enzymatic method, we can increase exam security without compromising its reliability and possibly reduce clinical patient discomfort.

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Performance evaluation of novel C-peptide immunoassay reagent using a fully-automated immunoassay analyzer

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Background: C-peptide is co-secreted with insulin in equimolar amounts from pancreatic β cells. Assessment of endogenous insulin production with C-peptide immunoassay requires sufficient sensitivity and high specificity. In this study, we evaluated the analytical performance of newly developed C-peptide reagent.

Methods: The ST AIA-PACK C-Peptide II reagent* on Tosoh AIA-2000 fully-automated immunoassay analyzer is an enzyme immunoassay which is performed entirely in a single cup. C-peptide in the sample is bound with monoclonal antibody immobilized on magnetic beads and alkaline phosphatase-labelled monoclonal antibody. After 10 minutes incubation at 37℃, the beads are washed to remove unbound materials and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate. The amount of enzyme-labelled monoclonal antibody that binds to the beads is directly proportional to the C-peptide concentration in the sample. A standard curve is constructed using the Calibrator Set and unknown concentration of C-peptide is automatically calculated using this curve. In this study we evaluated the precision, functional sensitivity, interference, recovery and cross-reactivity of this new reagent toward human proinsulin. Method comparison, against ARCHITECT C-peptide immunoassay based on chemiluminescent immunoassay, was evaluated with clinical specimens from patients. Correlation of the C-peptide
Low testosterone concentrations: only mass spectrometry?

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Background: total testosterone level measurement is the most requested one among steroid hormones assays. Unfortunately, the diagnostic accuracy at low concentrations of the most common immunoassays proved to be insufficient. In 2007 the Endocrine Society recommended the determinations of testosterone in children and in women has to be done only with one reference method (extraction, chromatography and determination by mass spectrometry). Due the related difficulties in most of the laboratories the testosterone determinations are still done by immunoassays.

Samples and methods: we measured testosterone with three different fully automated immunoassays present in most of the clinical labs and repeated the determinations both with a commercial RIA and LC-MS/MS method. The latter one, considered the reference method, has been done in the Perkin Elmer labs (Turku, Finland), with updated equipment, by skilled personnel and determinations carried out in replicated. The serum samples were collected from 70 patients, male and female in pediatric age. The obtained concentrations by LC-MS/MS, considered as reference, ranged from 11 to 110 ng/dL.

Results: the distribution of the concentrations obtained with the methods used should be noted that, although the averages and medians of the concentrations obtained with the LC-MS/MS method are less, the differences are not such as to distort the clinical information can be obtained: the 3 automated methods show values ranging from 10 to 134 ng/dL, with correlations coefficients respectively to LC/MS/MS ranging from 0,829 to 0,934; whereas the RIA method shows a higher concentration’s dispersal, values ranging from 20 to 149 ng/dL and a worse correlation to the reference method. Due the related difficulties in most of the laboratories the testosterone determinations are still done by immunoassays.

Conclusions: the position of the scientific community on total blood testosterone measurement at low concentrations is critical to the use of direct immunoassays because without the necessary diagnostic accuracy, and recommends the use of methods that are not within the reach of general laboratories. Our results, although preliminary, open an interesting perspective on the possibility of arriving at a reasonable future to employ even the immunoassays, certainly more feasible, as an aid to diagnosis of common and important endocrine syndromes of the woman and the child.

Quality control management improving immunoassays systems in the clinical laboratorial routine

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Background: automated analyzers provide several advantages on processing immunoassay methods. The literature demonstrates that laboratory errors can be associated with pre-analytical (30.6%), analytical (31.6%) and post-analytical (30.8%) processes or even due to combined processes (6%). Errors in the analytical phase are commonly related to lack of preventive maintenance, inappropriate Quality Control (QC) management, and improper handling of samples or reagents. Errors due to analytical problems have been significantly reduced over time, but there is evidence that this interference may have a serious impact on patient results, especially for immunoassays.

Objective: the aim of the study was to identify the potential causes for quality control variability in immunoassays to improve the laboratory routine productivity when adopting best quality control practices.

Methods: 32,760 quality control points of Immunoassay Plus QC Lot 40240 (BioRad) were collected during eight months using 14 ADVIA Centaur Systems (Siemens Healthcare Diagnostics), in the clinical laboratories. The statistics evaluated were: Coefficient of variation (CV), Standard deviation (SD), observed mean and outliers. Data was compared to those reported on Biorad International Quality Control Program. All assays that presented better or equal statistic results were considered acceptable. Assays with higher CV than the reference, BioRad worldwide report, were submitted to further technical investigation and corrective actions. After critical analysis of the first four months data, some improvements were implemented, such as: a new plan of the preventive maintenance, increasing periodicity from once per quarter to once each three months matching laboratory number of tests; a new definition of mean and standard deviation for each QC target level for each assay. Once improvements were implemented, quality control data was collected during the following four months.

Results: in the first four months period, before implementation of proposed improvements, the CV mean was 18.8%, 5,402 tests were spent in calibrations, 16,841 tests were used in QC material analysis and reagents profitability was 96.4%.

Classification of Children with New-Onset Diabetes Mellitus Using an Auto- Antibody Algorithm

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Background: historically the diagnosis of Type 2 diabetes mellitus (T2D) in children has relied on a typical clinical phenotype. The ability of experienced clinicians to correctly classify the type of DM based upon the clinical phenotype has, however, recently been challenged. Since the appropriate classification has important implications with regard to treatment options, expected outcomes and genetic counseling, a systematic, cost-effective algorithm to assist in the initial classification of DM is needed.

Objective: to evaluate the use of an auto-antibody algorithm to classify new onset diabetes patients and its use in curtailing testing costs.

Methodology: Data from children (<18 yrs of age) hospitalized at CCMC from Jan 1–Oct 2012 with new-onset DM was analyzed. In contrast to T2D, T1D is an autoimmune disease (AD) characterized by the presence of >1 diabetes-related antibodies (DR-Ab). Historically the initial evaluation, including DR-Ab testing, has been left to the discretion of individual Pediatric Endocrinologists. Other Abs are often measured to assess concurrent autoimmune diseases that commonly occur in individuals with DM, such as Hashimoto’s thyroiditis and celiac disease. Inclusion Criteria: 1) Age <18 yrs at diagnosis; 2) New-onset DM; 3) Onset Jan 2010–Oct 2012.

Results & Conclusions: The American Diabetes Association classifies DM into T1D, T2D, gestational diabetes, and diabetes due to other causes. While the majority of those <18 yr of age have T1D, the number with T2D is increasing. Individuals with T2D are often obese. With the exponential increase in the number of children who have become overweight/obese, classifying DM based on a child’s phenotype has become problematic. In children with overt signs/symptoms of DM the presence of ≥1 DR Ab is generally considered sufficient evidence of auto-immunity (i.e. T1D). In our study, subjects were routinely tested for 2 DR-Ab (GAD, ICA). Since 73.3% of subjects were positive for GAD, additional testing for ICA increased cost w/out additional benefit. While not included in the present study, additional screening tests are sometimes also requested for celiac and thyroid disease. For those whose initial screening was positive (celiac 12.5%; thyroid 16.5%), eliminating further testing would have helped reduce cost.

Ab testing to help classify children with new-onset DM may be enhanced with use of an algorithm, especially if it includes reflex testing. Reflex tests are tests automatically performed by the laboratory if the initial test requested fails to meet preset criteria. Subsequent tests can generally be performed w/out need for additional samples and may consist of ≥1 sequential tests. Although there is a charge for additional tests, if the likelihood of the criteria being met with the initial sample is high, reflex testing has the potential to reduce medical cost.


Endocrinology/Hormones

A Comparison of CVD risk in newly diagnosed hypothyroid and type 2 diabetes mellitus subjects using Framingham risk score sheet

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Background Thyroid disorders and type 2 diabetes are known for their association with CVDs owing to their effect on derangement of the lipid metabolism. However there are no studies to document a comparative CVD risk in these two disorders.

Aim We aimed to compare the various CVD risk parameters in thyroid disorder and type 2 DM subjects at the time of diagnosis.

Material and methods The study participants were 150 hypothyroid and 180 type 2 DM subjects reporting for the first time to our endocrinal clinics. The patients were selected on the basis of symptomatology, a TSH >5µU/ml and a FBS >126 mg/dl. All patients were evaluated for BMI, Blood Pressure, serum Insulin, HOMA-IR, Lipid profile, apo-B and A1. CVD risk was assessed using the Framingham risk score. Statistical analysis was done using the students t-test to assess significance.

Results At diagnosis the hypothyroid and T2DM subjects presented with raised BMI (p<0.001), hypertension (SBP 132.98 ±17.40 vs 132.60± 12.18 [NS]; DBP 86.52± 9.82 vs 88.79: 8.02 [NS]), insulin resistance (30.63±16.18 vs 17.29 ± 11.65 [p<0.0001]), gross dyslipidemia, with the T2 diabetic subjects showing significantly raised total cholesterol (231.15± 22.19 vs 213.50 ± 38.95, p<0.0001), triglycerides (197.35± 55.31 vs 187.91 ± 39.12, p<0.0001), reduced HDLc (33.56±2.67 vs 42.99 ±4.70, p<0.0001) and significantly reduced apo B (154.47± 12.87 vs 175.58 ± 34.56, p<0.0001) and apo A1 (96.94± 8.55 vs 139.76 ± 17.40, p<0.0001). The CVD risk ratios Tchol/HDLc 6.93 vs 5.03 and apoB/apoA1 were 1.60 vs 1.29. The ten year risk of CVD in the T2DM subjects was 25% and in the hypothyroid subjects was 13%.

Conclusion: The present study concludes a significantly raised CVD risk in T2DM as compared to hypothyroid subjects at diagnosis.

Serum ghrelin, obestatin and nesfatin1 levels in pregnant women with hyperemesis gravidarum

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Backgrounds: Hyperemesis gravidarum, which affects 0.3-2.3% of pregnancies, is a disease of severe nausea, vomiting, and anorexia in early pregnancy resulting in dehydration and weight loss. Prealbumin is an indicator to assess nutritional status, so our data also suggests that prealbumin levels are decreased in patients with Hyperemesis gravidarum. Ghrelin is involved in stimulation of appetite, control of energy balance, and gastric motility. Ghrelin administration increases food intake through central mechanisms. One possible explanation might be that the decreased levels of ghrelin in Hyperemesis gravidarum may be a mechanism to lose of appetite and the energy balance of the Hyperemesis gravidarum pregnant women.

Evaluation of the Impact on IGF-I Control of Pharmacological Treatment with Octreotide LAR isolated compared to Association with Cabergoline in Patients with Acromegaly

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Background: Discrepancies concerning GH and IGF-1 levels in acromegaly patients can occur in patients submitted to pharmacological treatment. Objectives: The aim of this study was to compare the efficacy in decrease and normalization of IGF-I values of Octreotide LAR treatment isolated and associated with cabergoline, based on IGF-I determinations.

Methods: This is a case series study that enrolled 34 patients with confirmed diagnosis of acromegaly recruited from outpatient clinics of the Neuroendocrine Unit of the University Hospital of Brasilia. All of them received the diagnosis of acromegaly confirmed by clinical findings suggestive of the disease, elevated GH and age-matched IGF-I levels, GH not suppressible by the oral glucose load and evidence of pituitary adenoma on CT or MRI. The statistical analysis was performed using SPSS 17.0 software. Values are expressed as the mean ± standard deviation. The values of IGF-I are presented both as absolute values as percentage values of the upper normal limit normal range of IGF-I (% UNLV-IGF-I).

Results: The cohort was composed by 15 men and 19 women; mean age 54 (27-74) years old, divided in two groups, group 1, treated by Octreotide LAR (OC-LAR) 30 mg/month, and group 2 treated by Octreotide LAR (OC-LAR) 30 mg/month associated with cabergoline 20 mg/week (OC-LAR + CGB). Mean serum IGF-I and % UNLV-IGF-I pretreatment were significantly higher in the group OC-LAR + CGB. Those variables decreased 6 and 12 months after treatment in both groups, and the values of OC-LAR-CGB group became inferior to those treated with OC-LAR group. However, no significant difference was found between the OC-LAR and OC-LAR + CGB group neither 6 nor 12 months after treatment.

Conclusion: OC-LAR + CGB association resulted in a significantly higher decrease of IGF-I, both 6 and 12 months after treatment, compared to those treated with OC-LAR.

ADVIA Centaur® Vitamin D Total Assay*: Expected Vitamin D Values in a Healthy Pediatric Population

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Background: Vitamin D is a hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. It is a key regulator of bone metabolism. Vitamin D, is derived from skin exposure to sunlight while food supplements contain either Vitamins D, or D. Vitamin D deficiency is caused by a lack of exposure to sunlight and by insufficient dietary intake. Measurements of serum or plasma levels of the metabolite 25-hydroxyvitamin D (25(OH)D) are the best indicators of nutritional Vitamin D status.
Objective: The goal of the study was to test healthy pediatric donor specimens in order to establish pediatric observed values for the ADVIA Centaur Vitamin D Total assay.

Methods: Serum samples were obtained from donors with ages ranging from 1 year up to 21 years. Donors resided in 8 regions, geographically distributed across the continental U.S. Donations occurred throughout one calendar year. All donors were free of chronic or active diseases, and were not receiving any prescription medications within 7 days of donation. All samples were assayed for iPTH and TSH on the Siemens IMMULITE® 2000 Immunoassay System. Samples with abnormal iPTH or TSH levels were excluded. The remaining samples were assayed for total vitamin D Total on the Siemens ADVIA Centaur. The ADVIA Centaur Vitamin D Assay used was aligned to the ID-LC/MS/MS 25(OH)vitamin D Reference Measurement Procedure (RMP), the reference procedure for the Vitamin D Standardization Program (VDSP).

Results: After all inclusion criteria were met, 227 samples were assayed for Vitamin D Total. Values were calculated for each age group, by season, and by geographic location. The lower and upper reference limits were estimated as the 2.5th and the 97.5th percentiles of the distribution of test results for each age group. The n, mean, median, 2.5th and the 97.5th percentiles for each population sub-group were:

- Northern: n=119, mean=23.55, median=22.17, 25%=12.36, 75%=38.92
- Southern: n=108, mean=26.33, median=25.47, 25%=9.70, 75%=49.16
- Summer: n=136, mean=26.64, median=24.86, 25%=12.46, 75%=46.58
- Winter: n=91 , mean=22.23 , median=22.10 , 25%=9.70 , 75%=32.38
- 1yr-3yr: n=22 , mean=23.34 , median=24.41 , 25%=13.98 , 75%=32.45
- 3yr-12yr: n=114 , mean=24.99 , median=23.10 , 25%=12.46 , 75%=45.96
- 12yr-21 yr: n=91 , mean=25.09 , median=23.95 , 25%=8.16 , 75%=45.83

Conclusion: Vitamin D levels were consistent across the 3 age groups with no apparent changes with age. There was no statistical difference between those receiving and not receiving vitamin supplements. Vitamin D levels were statistically higher for the southern region versus northern region, and for summer (maximum sunlight) versus winter (minimum sunlight). There was extensive overlap in ranges for all sub-populations and the expected range for the entire population can applied to any of the sub-populations.

* This version of the ADVIA Centaur Vitamin D Total assay is not available for sale in the U.S.

Product availability varies from country to country and is subject to local regulatory requirements.

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Glutamate Decarboxylase Antibody Positivity in Diabetics

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Background: Glutamate decarboxylase antibody (GAD) testing is useful in identifying patients with latent autoimmune diabetes in adults (LADA), and some studies showed that higher antibody titres were associated with specific phenotypes. We looked at patients with positive GAD and reviewed their laboratory and clinical features.

Methods: All GAD tests performed in 2013 were included, and positive results were analyzed with respect to demographic parameters, indication for testing (evaluation of diabetes mellitus or neurological signs or symptoms), presence of other autoantibodies or autoimmune diseases. GAD was performed using radioimmunoassay using the CentAK® kit and a positive result was defined as ≥9U/L. Statistical analysis was done using SPSS Version 17.0.

Results: There were 454 GAD requests in 2013, with 75(16.5%) positive cases. The median age was 39.3 years old, with female: male ratio of 0.55.

In patients with positive GAD, the median age was 43.4 years old, with female: male ratio of 1.06. There were 45 Chinese, 10 Indians, 9 Malays and 10 from other ethnic groups. 94.6% were requests to exclude LADA, and 21.3% had concurrent requests for anti-islet cell antibody, with 50% positivity. 4 patients had co-existing autoimmune diseases (myasthenia gravis, thyroid disease with positive thyroid peroxidase antibody, vitiligo and pernicious anemia).

GAD titers showed a Gaussian distribution with a left skew and peak at 0-25U/L. There was no association between GAD titres and age or C peptide levels.

Conclusion: GAD levels were predominantly ordered to evaluate diabetes mellitus in younger patients and there was an association with anti-islet cell antibody and other autoimmune diseases. Further studies may be performed to determine the clinical significance of high GAD titres in diabetics.

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**A-170**

New therapeutic effect of metformin due to increased levels of FGF21?

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Background: Fibroblast growth factor 21 (FGF21) is an endocrine hormone that exhibits anti-obesity and anti-diabetes effects. Recently was presented that metformin in patients with type 2 diabetes modulates FGF21 expression and blood concentration.

Results indicate that metformin induced expression of FGF21 through an ATP4-dependent mechanism by inhibiting mitochondrial respiration independently of AMPK and it’s concentration in blood. AIM: Studying the effect of metformin on the concentration of FGF21 in serum in type 2 diabetes patients.

Methods: The study was approved by the Ethics Commission of the Hospital Starnberk. Study was monocentric, prospective and randomized. A total of 108 individuals were recruited for our study (18 healthy controls (HC), 18 T2D individuals without anti-diabetes therapy (W), 18 T2D individuals with diabetes monotherapy with derivate of sulfonylurea (SU), 18 T2D individuals with diabetes monotherapy 500 mg metformin/day (M5), 18 T2D individuals with diabetes therapy 1000 mg metformin/day (M10), 18 T2D individuals with diabetes therapy 1500 mg metformin/day (M15). Anthropometric (height, weight, BMI, waist circumference (WC)), clinical (systolic and diastolic pressures) and laboratory fasting analyses were performed. Serum samples were separated in a cooled centrifuge and immediately analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, glucose, high sensitivity CRP, creatinine, uric acid, AST, ALT (all Siemens, Advia 1800). FGF21 serum level was determined by a commercially available ELISA kit (Biovendor, DS2, Dynex) in serum samples stored at -80°C.

Results: The study analysed 108 subjects, of which 18 were in good health while 100 probands suffered from T2D. In defined subgroups, we found no significant differences by gender in FGF21 concentration. Healthy individuals had the lowest
values of FGF21, in other subjects are increased by the value of the diagnosis, the type of therapy and dose (HC 84.2 ng/l vs W 111.6 vs. SU 158.6 vs. M5 269.7 vs. M10 404.1 vs. M15 558.7, P <0.01). Changes remained significant after adjustment for age, sex and BMI. Serum glucose levels fluctuated in subgroups below 8 mmol/l.

**Conclusion:** in a randomized prospective study, we for the first time confirmed the recently presented hypothesis that metformin leads to the rise of FGF21. The new finding was the fact that this happens regardless of gender, weight and age of probands. FGF21 induction by metformin might explain a portion of the beneficial metabolic effects of metformin.

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**A-171**

**Pre-analytical assessment of AMH stability in human serum using a well-characterized midpro-mature immunoassay**

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**Background:** The aim of the study was to assess the stability of AMH in human serum using a well-characterized midpro-mature immunoassay.

**Relevance:** AMH is a homodimeric glycoprotein composed of two 55 kDa N-terminal and two 12.5 kDa C-terminal homodimers, non-covalently linked by disulphide bridges. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. To date, no publication has clearly stated if the AMH variability is related to process (pre-analytical) or the assay. The AMH in female serum is mostly pro-mature associated form. The kinetics of association of pro and mature is rapid. Assay design that includes stable epitope antibodies and is not impacted by molecule association or complements will generate reproducible results.

**Methods:** A prospective study (n=16) was designed in which serum samples were tested within 3 hrs of draw, aliquoted and stored at room temperature (RT), -20°C, 2-8°C and re-assayed at 7, 10, 24, 48 and 168 hours. Multiple samples were thawed up to 4 cycles and measured at two independent sites. A well-characterized two-step, ELISA (Ansh Labs, US AMH, AL-105) was used to measure AMH levels in 25 μL of sample in <3 hours. The assay is specific for human and measures pro-mature AMH complex. The assay is calibrated (0.09-19.4 ng/mL) against standardized recombinant human AMH.

**Results:** No significant changes were observed when samples were stored at RT, 2-8°C and -20°C. The median AMH concentration (16 serum samples, range 3.1-71 ng/mL) measured at 7, 10, 24, 48 and 168 hrs were 5.2, 4.9, 4.9, 5.0 ng/mL at RT, 5.0, 5.0, 4.7, 4.3, 4.4 ng/mL at -20°C and 4.8, 4.8, 5.0, 4.7, 4.9 ng/mL at 2-8°C. The average CV on multiple runs at RT, 2-8°C, -20°C was 8.7%, 6.9%, 9.3%, respectively. Total imprecision was expressed as coefficient of variation (CV%). Patient samples from healthy subjects, hypo and hyperthyroidism were freshly collected from the clinical laboratory of our hospital. The comparison studies were performed using CL 2000i and the reference methods in our laboratory, Siemens ADVIA Centaur and Beckman Coulter DxI 800.

**Results:** The CVs of TSH, FT4, FT3, TT4, and TT3 are in a range from 0.748 to 1.051 and the intercepts from -0.48 to 0.6. All of the five assays displayed high interassay correlation (r² > 0.92). TSH displayed the highest correlation between CL 2000i system and Centaur XP system (slope = 0.993; r² = 0.971), while TT4 showed the lowest agreement between CL 2000i system and the Centaur XP (slope = 1.051; r² = 0.921).

**Conclusion:** The imprecision was highly acceptable for all the five parameters of thyroid hormones tested. The method comparison between CL-2000i system and the reference methods evidenced high concordance. Therefore, the parameters of CL-2000i system are well suited for the detection of thyroid hormones in clinical laboratories.

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**A-172**

**Performance of Thyroid Hormone Assays on Mindray CL-2000i Chemiluminescence Immunoassay System**

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**Background:** Thyroid hormones are among the first-line tests subject to international collaborative investigations, aiming at assessing the key performances and comparability of results between the available tests. Mindray CL-2000i Chemiluminescence Immunoassay System (CL-2000i) is a recently launched automatic immunoassay system. We have evaluated the performance of the system with serum thyroid-stimulating hormone (TSH), free thyroxine (FT4), free triiodothyronine (FT3), total thyroxine (TT4), and total triiodothyronine (TT3).

**Methods:** Imprecision studies were performed according to the CLSI EP5-A2 guideline, and have been evaluated using two samples with low and high concentration. The within run imprecision was performed by measuring each sample for 20 times. The total imprecision was evaluated by measuring each sample continuously for 20 days with the same lot of reagent. The imprecision was expressed as coefficient of variation (CV%). Patient samples from healthy subjects, hypo and hyperthyroidism were freshly collected from the clinical laboratory of our hospital. The comparison studies were performed using CL 2000i and the reference methods in our laboratory, Siemens ADVIA Centaur and Beckman Coulter DxI 800.

**Results:** The CVs of TSH, FT4, FT3, TT4, and TT3 are in a range from 1.09 - 6.16% for within run, and from 3.22 - 9.16% for total imprecision. TT4 shows relative higher CVs for both within run and total imprecision comparing to other parameters, but within the manufacturer’s claim (≤10%). The comparison studies indicated slopes for the five thyroid hormone parameters ranged from 0.748 to 1.051 and the intercepts from -0.48 to 0.6. All of the five assays displayed high interassay correlation (r² > 0.92). TSH displayed the highest correlation between CL 2000i system and Centaur XP system (slope = 0.993; r² = 0.971), while TT4 showed the lowest agreement between CL 2000i system and the Centaur XP (slope = 1.051; r² = 0.921).

**Conclusion:** The imprecision was highly acceptable for all the five parameters of thyroid hormones tested. The method comparison between CL-2000i system and the reference methods evidenced high concordance. Therefore, the parameters of CL-2000i system are well suited for the detection of thyroid hormones in clinical laboratories.
Calcitriol and its free and bioavailable fractions are better markers than 25 hydroxy vitamin D for monitoring vitamin D status during pregnancy

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Objective: To compare bioavailable 25-hydroxy vitamin D (25OH-D) concentrations with those of bioavailable calcitriol (25 di Hydroxy vitamin D) in sera of pregnant women.

Relevance: Pregnancy is associated with major changes in calcium homeostasis with critical roles played by vitamin D and its metabolites. Current practice favors monitoring of vitamin D status in pregnancy using 25OH-D concentrations in serum. Calcitriol is the active form of vitamin D. The level of 25OH-D and calcitriol vary due to changes in maternal serum vitamin D binding protein (DBP). Serum concentrations of bioavailable and free vitamin D are not influenced by DBP. Therefore, we measured total, bioavailable and free fractions of 25OH-D or calcitriol in serum samples of pregnant and non-pregnant women. Correlation between total 25OH-D and its fractions (bioavailable and free) and calcitriol and its fractions with PTH and CTX (terminal collagen degradation product) was explored in order to determine which compound or compounds were optimal markers of vitamin D status in pregnancy.

Methods: Bioavailable 25OH-D or bioavailable calcitriol are fractions, not bound to DBP. They are the combined fractions of albumin bound and free fractions of 25OH-D or calcitriol. To obtain the bioavailable fraction, total vitamin D (25OH-D or calcitriol) was quantitated using immun assays (IDS, Phoenix, AZ). DBP was quantitated by an ELISA using reagents from R&D systems. Albumin was quantitated by a colorimetric method. Using the affinity constants of 25OH-D and calcitriol for DBP, and the affinity constants of 25OH-D or calcitriol for serum albumin, bioavailable 25OH-D/bioavailable calcitriol, free 25OH-D and free calcitriol were calculated.

PHT and CTX assays were performed in pregnancy serum samples using IDS kits. Pregnant serum samples (n=54) were collected between 27 to 38 weeks of pregnancy.

Results: Total 25OH-D was significantly lower in pregnant women despite a significant increase in DBP (276±15 Vs 410±30 µg/mL) bioavailable and free 25OH-D levels were lower than normal (n=55) and were in the deficiency or insufficiency range. Although the levels of PHT and CTX were in the normal range. The correlation between PHT with total 25OH-D was poor (r=0.3). There was also poor correlation between the PHT and bioavailable or free 25OH-D (r=0.5). Calcitriol was high in the pregnancy samples (127.5±15.5 µg/mL) compared to non-pregnant samples (36.2 ± 5.6 µg/mL) and the DBP-corrected bioavailable and free calcitriol was twofold higher than non-pregnant controls. Calcitriol and its fractions (bioavailable and free of calcitriol) correlated well with serum PHT and CTX (r>0.9). Conclusion: Current assessment of vitamin D status in pregnant women by measurement of 25OH-D does not adequately reflect calcium homeostasis in pregnancy. 25OH-D or its fractions do not correlate with PHT or CTX. On the other hand, calcitriol correlates well with PHT and CTX in pregnancy when determined either as total, bioavailable or free calcitriol. The data indicate that bioavailable or free calcitriol are the best markers for determining vitamin D status in pregnant women.

A-175

Analytical Measurement Range (AMR) Monitoring for Immune-hormone System

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Background: Linearity tests verify if a method is able to provide linear results, proportionally to the analyte’s concentration. It is extremely important to ensure the accuracy of the results released. A Brazilian laboratory established a self-inspection program to verify the efficiency and accuracy of procedures and results through the implementation of the Easy Linearity Curve (ELC) statistic tool to monitor an immune-hormone system. ELC checks assay linearity and provides additional safety and reliability to their results. This study aims to use the statistic tool to monitor the AMR of ten immune-hormone parameters.

Materials and methods: Samples were selected from the laboratory routine, eliciting concentrations within assay linearity range for each test. Then, samples were tested for Insulin-like Growth Factor 1 (IGF-1), Insulin-like Growth Factor Binding Protein 3 (IGFBP-3), Growth Hormone (GRH), Prolactin (PRL), Sex Hormone Binding Globulin (SHBG), C-Peptide (Pp-C), Homocysteine (HCY), Adrenocorticotropic Hormone (ACTH) and Intact Parathyroid Hormone (iPTH) on IMMULITE 2000 System (Siemens Diagnostics Healthcare) and results analyzed with the ELC tool.

Results and Discussion: AMR studies were carried out according to CLSI Guideline EP6-A. Results are shown in the following table below. Tested Samples showed satisfactory linearity results for each parameter. The coefficients of second and third degree regression are statistically equal to zero, at 5% of the significance level. All results analyzed by ELC, presented Assay Linearity Range as established by the manufacturer, within CLSI Guideline EP6-A and Total Error Laboratory’s target.

Conclusion: We conclude that the implementation of a self-verification program as ELC can increase efficiency and accuracy of procedures and results; aiding laboratory on accomplishing Quality Control Program requirements and guaranteeing safety and reliability of their released results.

Table 1 -

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Clinical Applications of Adiponectin Measurements in Type 2 Diabetes Mellitus - Screening, Diagnosis and Marker of Diabetes Control

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Background: Adipose tissue-derived adiponectin has pleiotropic protective effects with suppression of inflammatory and metabolic derangements that may result in insulin resistance, metabolic syndrome, Type-2 diabetes (T2DM) and cardiovascular disease. No study has evaluated the potential clinical significance of adiponectin measurements that may be useful in routine practice. The aim of this study was to evaluate adiponectin as a screening tool and diagnostic marker of T2DM and diabetic control.

Methods: Fasting adiponectin, insulin and glucose and HbA1c were determined in 575 subjects with undiagnosed diabetes but with family history of T2DM. To evaluate adiponectin as a marker of DM control, we studied 776 patients with known T2DM duration of 12.4 ± 8.1 years. Clinical and anthropometric data were recorded and subjects were classified on the basis of the degree of adiposity, insulin resistance (IR) using the homeostasis model assessment, and achievement of target HbA1c levels <53mmol/mol. Using standard cut off values for glucose and HbA1c, receiver operating characteristic curve (ROC) analysis was used to examine the diagnostic performance characteristics for undiagnosed DM.

Results: In undiagnosed subjects, adiponectin was significantly lower in subjects with IR (7.0 vs 8.5 µg/mL) and diabetic subjects (7.4 vs 8.6 µg/mL) compared with those without. 73 of 575 subjects were found to have T2DM. Binary logistic regression showed that the odds ratio of T2DM as predicted by adiponectin was 0.88 [95% confidence interval 0.80-0.96; p = 0.007]. At cut-off points of 7.5 µg/mL, the diagnostic sensitivity and specificity of adiponectin were 94% and 51% respectively. Using the ADA glucose and HbA1c diagnostic criteria as reference, the area under the adiponectin ROC curve for diagnosis of DM was 0.74 (95% CI 0.70 - 0.91). In known T2DM subjects, those with good control (HbA1c < 55mmol/mol) had significantly higher adiponectin (8.5 vs 7.1 µg/mL) compared to subjects with poor control.

Conclusions: Adiponectin levels are associated with better glycemic control and could be useful adjunct for screening for IR and T2DM. Therapeutic measures that increase adiponectin levels might be valuable targets for improving diabetes control and decreasing complications.

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Performance of GHR stimulation tests at a private laboratory in Brazil: Is insulin tolerance test still the best?

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Background: Growth hormone deficiency (GHD) is the most important endocrine cause of short stature in childhood. Once growth hormone (GH) secretion is pulsatile, diagnosis of GHD is made with a combination of clinical assessment, IGF1 and
IGFBP3 levels, and GH stimulation tests. In Brazil, the most common GH stimulation tests are insulin tolerance test (ITT), clonidine and glucagon. The aim of this study was to determine the performance of those GH provocative tests and to make comparisons among them and with IGF1 levels.

Methods: Retrospective assessment of GH stimulation tests, performed during the year of 2013 in children between 4 to 18 years old, including 141 ITT (mean age 11.72 2.58 yrs, 72.5% male), 285 clonidine tests (mean age 10.41 2.91 yrs, 76.5% male) and 42 glucagon tests (mean age 7.00 3.4 yrs, 66.7% male). The mean dose of each medication administered was respectively 0.04U/kg, 0.138 mg and 0.568 mg and the mean glucose nadir at ITT was 27.95 8.02. Comparison among tests showed statistical difference regarding peak stimulated GH (GH > 5.0 ng/mL) between ITT and glucagon (p<0.01) and ITT and clonidine (p<0.01), but not between glucagon and clonidine tests (p=0.104). Peak stimulated GH happened at 26.24% (mean GH 10.62 5.78), 82.10% (mean GH 11.88 5.79) and 71.43% (mean GH 11.63 7.38) from ITT, clonidine and glucagon tests, respectively. GH peaks concentration was at time of hypoglycemia at ITT (70.6%), at 60 minutes after stimulation with clonidine (60.3%) and at 2h after stimulation with glucagon (63.3%), respectively. Levels of IGF1 did not correlate with GH answer to the stimulation at insulin (p=0.6165), at 60 minutes after stimulation with clonidine (0.4914) and glucagon tests (p=0.5551).

Conclusion: Our finding that clonidine and glucagon tests presented a better rate of response compared with ITT represents a new scenario for GH provocative tests, once ITT has been considered the gold standard test for GHD investigation. Maybe it could be explained by the fact that in a private laboratory environment, we can not let the patient recover spontaneously, which could increase side effects. Instead, the recovery from hypoglycemia is done with oral glucose, which may impair the GH peak, finding reported by Yeste and cols. Hence, clonidine and glucagon tests emerge as a reliable and safer alternative to ITT. Surprisingly, IGF1 levels did not correlate with rates of GH response to the tests, which highlights the importance of a clinical/laboratory combined evaluation.

A-180
A Fully-Automated 1,25-Dihydroxy Vitamin Dxp Assay on the IDS-iSYS Automated System

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1,25-Dihydroxyvitamin D (1,25D) is one of the major regulators of calcium metabolism. Due to its lipophilic nature and low circulating concentration, the measurement of 1,25D concentration levels has been labour intensive and technique dependent in addition to multiple equipments required for the sample purification procedure. We reported the results of fully automated IDS-iSYS 1,25 VitD assay. IDS-iSYS 1,25 VitD assay purifies 1,25D in human sera utilising the anti-1,25D antibody coated magnetic particles in cuvette 1. After incubation, the magnetic particles are washed and 1,25D is eluted. The eluate is transferred to cuvette 2 where the immunoassay procedure is performed utilising the IDS-iSYS 1,25-Dihydroxy Vitamin D test. The purified 1,25D competes with 1,25D-Acridinium (1,25D-ACR) for a limited amount of biotinylated anti-1,25D antibody sites. Bound complexes are captured via streptavidin-coated magnetic particles. Following washing, the bound 1,25D-ACR is measured where signal generated is inversely proportional to the 1,25D in the samples. Each assay was performed in duplicate, and intra- and inter-assay coefficients of variation were determined for each concentration of 1,25D. The data shows that for normal samples, performance of the Pointe Scientific, Cantor, MI

Keywords: cortisol, stress, firefighters.

A-182
Evaluation of a direct HBAlc assay on a fully automated chemistry analyzer versus two common HPLC methods.

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Pointe Scientific, Inc. (now part of MedTest) has developed a latex-enhanced immunoturbidimetric assay that directly measures the % HbA1c in whole blood. This new method was adapted to a Roche Hitachi 917 clinical chemistry analyzer in a fashion that offline sample pretreatment was not required. The objective of this study was to evaluate the performance of this direct HBAlc assay on the Hitachi 917 chemistry analyzer versus a Tosoh G8 and a Bio Rad Variant II (HPLC methods) at a large commercial lab. Samples with normal hemoglobin that were run on the Tosoh G8 versus the Hitachi 917 showed a correlation of R=0.987, with a regression equation of y = 1.15x – 0.80. (n=172). Additional samples containing various hemoglobin variants were compared to the results from the Biorad and Tosoh systems. Utilizing all samples (normal and variants) resulted in a correlation of R=0.958, with a regression equation of y = 1.15x – 0.77. (n=256). High and low Biorad QC materials were run for Day-to-Day and Within Run precision: Results are shown below:

<table>
<thead>
<tr>
<th>Day to Day Precision</th>
<th>Within Run Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average: 1.83 0.21</td>
<td>Average: 1.75 0.27</td>
</tr>
<tr>
<td>Std Dev: 0.144 0.166</td>
<td>Std Dev: 0.165 0.082</td>
</tr>
<tr>
<td>CV: 7.7% 0.9% 5.6%</td>
<td>CV: 3.47 0.9% 0.89</td>
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</table>

The data shows that for normal samples, performance of the Pointe Scientific HBAlc assay is comparable to the Tosoh HPLC system. When Biorad and Tosoh variant sample results were included, the correlation was minimally affected. These results demonstrate that this assay can be a viable solution for large volume testing environments where HPLC systems may not be capable of handling a very high volume of samples.
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Analytical evaluation of a glycated protein method on the Siemens ADVIA 1800

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Background HbA1c has become the gold standard for assessment of glycemic control in individuals with diabetes mellitus. However the test cannot be used if the individual has a homozygous hemoglobin variant or a condition in which there is a rapid turnover of red blood cells. In these cases glycated protein has been proposed as an alternative assay. We wished to evaluate the analytical performance of the Diazyme Glycated Protein assay, establish a reference interval and to compare the glycated protein results against HbA1c results.

Methods The Diazyme Glycated Protein assay was programmed onto a Siemens ADVIA 1800 analyzer using a program supplied by the reagent manufacturer. Glycated protein was analyzed on samples that had serum albumin, total protein and HbA1c results available. Samples with a bisalbumin were also analyzed for glycated protein by 2 methods.

Results The within run imprecision was calculated at 1.2% and 0.4% respectively at glycated protein concentrations of 181.2umol/L and 684.2 umol/L. At the same concentrations the between run imprecision was 2.3% and 1.2% respectively. Correlation of glycated protein against HbA1c gave a regression equation of y (glycated protein) = 56.41(HbA1c)-35.72, (n=155, r2=0.91) The reference range was calculated on 42 samples that had both glucose and HbA1c within the respective reference intervals and was 165 (90% CI 145 to 186) to 367 (90%CI 340 to 386) umol/L. The correlation of the manufacturer’s suggested reference intervals of 100 to 295 umol/L. Using the ratio glycated albumin /albumin the reference interval was 10.5 (90%CI 9.8 to 11.4) to 18.5(90%CI 17.5 to 19.4). Linearity was established from 10 to 1150 umol/L. Comparison of Diazyme Glycated Protein results on bisalbumin samples with a glycated albumin method, Lucica GA-L, gave a correlation equation of y(Diayme)=0.5 (Lucica GA-L) +6.2 (n=25), r=1, two tailed T test p<0.14.

Conclusion The analytical performance of the Diazyme Glycated Protein was satisfactory although the reference interval obtained was higher than that suggested by the manufacturer. Comparison against a glycated albumin method for bisubnumia samples was good although the absolute values are different. The effect of albumin variants needs to be further evaluated.

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Hypercalcemia with Normal PTH: A Diagnostic Puzzle?

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Introduction The two major causes of hypercalcemia are primary hyperparathyroidism and malignancy, where patients generally have elevated PTH results in the former and suppressed PTH in the latter condition. However, there is a smaller group of patients who present with mild hypercalcemia and normal PTH that can be a diagnostic puzzle, if clinical history and condition are unremarkable.

Objective: To determine the prevalence of hypercalcemia with normal PTH in a large endocrinology practice in an integrated health system and to determine what effect correcting (adjusting) the calcium concentration for albumin would have on the classification of hypercalcemia.

Results/Discussion: 3,958 calcium results were retrieved by a computer search of the data base from the endocrinology faculty practice at North Shore-LIJ Health System in Long Island, NY from January 2013 through January 2014, where 155 (3.9%) of these results have been classified as hypercalcemic, i.e., above 10.5 mg/dL. This study focused on calcium, PTH and albumin results without any patient history or other clinical information. Within this original hypercalcemic group a subgroup of 42 (27.1%) patients were identified with normal PTH results. For this subgroup albumin results were also retrieved and a corrected calcium concentration was calculated according to a standard textbook equation; Ca (corrected) = Ca (measured) + 0.8 x (4.0 – Albumin), where the calcium and albumin units of measure are mg/dL and g/dL, respectively. From the original subgroup of 42 patients, 18 (42.9%) were reclassified to normocalcemia based on corrected calcium results whereas 24 (57.1%) remained in the hypercalcemia classification. While the correction of calcium for albumin is not routinely performed or reported in most laboratories, its primary application has been for patients with hypoalbuminemia and not for patients with normal or high normal albumin as in this subgroup. However, the fact that a corrected calcium concentration reclassifies nearly half of these patients as normocalcemic gives rise to questions regarding the original classification of hypercalcemia based on total calcium concentrations. While measurement of free (ionized) calcium is considered a more accurate assessment of calcium status than total calcium, most laboratories primarily perform total calcium measurements for outpatient since this assay is easily automated and provides rapid, cost effective results. Outreach physicians do not often order free calcium because it is more expensive, has special requirements for blood collection and is usually not required for diagnosing calcium abnormalities. However, corrected calcium results are an estimation and free calcium measurements should be performed if the total calcium measurement is in question.

Conclusions: We have investigated a subgroup of hypercalcemic patients that have normal PTH results and have found that nearly half (42.9%) are reclassified as normocalcemic when the total calcium concentration is corrected for albumin. Since corrected calcium concentrations are an estimation it is suggested that measurement of free calcium may provide a more accurate assessment of calcium status for some patients in this subgroup.

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Validation of a productive platform for HbA1c testing

DASA, Barueri, Brazil

Background: The current guidelines for diabetes have recommended the use of HbA1c testing for diagnosis as well for monitoring of diabetes mellitus type 2. In HbA1c testing has an important role of diagnosis, there is the need for accurate and precise methods for quantification as recommended by the National Glycated Hemoglobin Standardization Program (NGSP). The purpose of exceeding the expectations of customers makes the labs have to consolidate their growth strategies and strengthen its activities in order to deliver results quickly, with quality and updated methodologies. Large routines demand faster methods which are efficient and have unquestionable quality results. DASA’s routine faces an average of 917,000 tests per month where we need to ensure that the time spent for tests processing do not generate a negative impact on laboratory routine, while guaranteeing that the integrity of the samples and the results of high quality promote the positive impacts within established time to deliver results. This study aims to validate a platform which improves productivity, reduces the time for analysis of samples and the number of installed equipments, enables the allocation of resources and maintains acceptable correlation with the methodology currently used for HbA1c testing.

Methods: HbA1c for Siemens ADVIA 2400 Chemistry® is a turbidimetric assay with range from 2.9% to 15.4%. To assess the assay’s correlation, we analyzed 100 whole blood samples collected in EDTA K2 tubes. The samples were divided according to the following three ranges: 4 to 6%, 6 to 14% and >15%. The results were compared with results obtained from Tosoh G7® platform. To compare the productivity, time to analyze the samples and TAT (Turn Around Time) were used 5 days of LIS data for routine performed at Tosoh G7® and a second period held on ADVIA 2400 Chemistry®.

Results: The comparative analysis of the results revealed a correlation coefficient (r) of 0.98 for HbA1c in ADVIA® 2400 Chemistry and a linear regression equation y = 0.9808 x + 0.2838 and R2 = 0.9579. When we verified LIS data analysis, the total test measuring time was 10.6 hours for a routine of 7,422 samples on five platforms Tosoh G7® and 4 hours for a routine of 8,550 samples on one platform ADVIA 2400 Chemistry®. The TAT for ADVIA Chemistry 2400® was 06 hours and 33 minutes and the Tosoh G7® was 20 hours and 32 minutes.

Conclusion: In the present study, Siemens HbA1c assay for the ADVIA Chemistry System has a proper correlation with the results of the Tosoh G7®. Thus, it ensures that the migration of this assay to tested platform causes no significant difference in results and clinical conduct. Furthermore, ADVIA Chemistry® 2400 presented higher throughput and a 68% reduction of TAT compared to the Tosoh G7®.

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The correlation of Fasting and Post Prandial Plasma Glucose with Estimated Average Glucose Levels

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Background: Association of fasting plasma glucose (FGP) and post prandial plasma glucose (PPG) on hemoglobin glycation is still controversial. Estimated average glucose (eAG) is a value calculated by hemoglobin A1c (HbA1c) that represents the integrated values for glucose over the preceding 8-12 weeks. The ‘A1C-Derived Average Glucose (ADAG)’ study showed a linear relationship between HbA1c and
**A-187**

Development of quantitative Estradiol assay for fully automated analyzer LUMIPULSTM G1200


Background: Estradiol (E2) is one of a female steroid hormone which is produced in ovarian tissue. Mainly it is used for monitoring of ovarian hypo-function or infertility treatment. In this time, we developed new reagent (Lumipulse G E2-III) which has excellent correlation with ID-GC/MS and reference materials (IRMM) and improved cross reactivity to some drugs or E2 derivative. Lumipulse G E2-III is one-step immunoassay, and E2 in specimen samples and ALP-labeled E2 competitively react with anti E2 monoclonal antibody coated on the micro particles. It is finally detected based on CLEIA technology. Here we show the excellent fundamental performance with fully automated chemiluminescence analyzer LUMIPULS1200.

Methods: The sample types used for this study were serum or Heparin-L. Correlation with ID-GC/MS, commercial competitive kit, matched pair correlation between serum and plasma, cross-reactivity to drugs, within-run precision, limit of detection (LoD) and 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 LUMIPULS G1200 (FUJIREBIO INC.).

Results: Correlation with ID-GC/MS with 25 specimen samples was excellent (slope: 1.04, regression: 1.00) and the measurement value in Lumipulse G E2-III calibrators was traceable to BCR577 reference materials. The significant correlation with the commercial available kit with 79 specimen samples was observed (Cobas, slope: 0.93, regression: 1.00, Centaur, slope: 1.06, regression: 1.00). Correlation between serum and heparin-L with 56 matched pair samples was excellent (slope: 1.03, regression: 1.00). Within-run precision % CVs for our assay ranged from 1 to 3% when 3 different levels of samples were tested, the calculated LoD at is 15 pg/mL and the LoQ ranged from 13 pg/mL to 17 pg/mL. As a result of evaluation with total 43 kinds of drugs and E2 derivatives, cross reactivity with almost all cross reagents were < 0.1 %.

Conclusion: These results demonstrated that improved Lumipulse G E2-III is a precise and highly useful for measuring E2 in serum and heparin-L. Also this assay is perfectly traceable to ID-GC/MS and reference materials.

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An Evaluation of Three Novel Biomarkers: Total-sLHCGR, LH-sLHCGR and hCG-LHCGR.

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Background: Researchers in the field of human reproduction need better research tools for the care of pregnant woman (e.g., prenatal diagnosis) and women who wish to become pregnant (e.g., fertility treatment). The assays for the soluble receptor for both human chorionic gonadotropin (hCG) and human lutetizing hormone (hLH) and the same soluble receptor bound to either hCG or hLH could be used as such research tools. The receptor is called the soluble LHCGR or sLHCGR. Three different ELISA assays have been developed and based on the type of HRP-labeled detector antibody employed, the user can quantitate Total sLHCGR, a complex of LH with sLHCGR or a complex of hCG with sLHCGR in a standard microtiter plate format. Methods: The levels of Total-sLHCGR, LH-sLHCGR or hCG-LHCGR were measured using sandwich ELISA. The three ELISA formats are the same; samples were diluted 5-fold, incubated for 10 minutes in a microtiter plate coated with a monoclonal capture antibody directed against the receptor. Next the HRP-labeled conjugate was added and incubated for 1.5 hour to generate the sandwich. After a wash step the substrate was added and after 20 minutes the reaction was stopped and read at 450 nm using a microplate reader. The optical densities from the reader are directly proportional to the amount of Total-sLHCGR, hCG-sLHCGR or LH-sLHCGR present in the sample. Results: The analytical sensitivity for the assays are 0.01, 0.004, and 0.02 Pmol/mL for the Total-sLHCGR, hCG-sLHCGR and LH-sLHCGR, respectively. The within assay precision was done at three different levels for the three assays (n=16). For hCG-sLHCGR the CV% are 4.4, 3.5, and 3.5% at 2.1, 11.7 and 18.0 Pmol/mL, respectively. For LH-sLHCGR the CV% are 3.2, 3.7, and 3.5% at 2.1, 11.2 and 28.9 Pmol/mL, respectively. For Total-sLHCGR the CV% are 7.4, 6.5, and 9.0 % at 3.5, 4.3 and 11.5 Pmol/mL, respectively. Inter-assay precision was determined for the three assays (n=4) and were for the hCG-sLHCGR 2.7, 7.7 and 4.2% at 2.2, 12.7 and 19.1 Pmol/mL, respectively. For LH-sLHCGR the CV% were 3.5, 3.6, and 2.9% at 2.1, 11.6 and 30.3 Pmol/mL, respectively. For Total-sLHCGR the CV% were 8.9, 8.6, and 11.0% at 3.7, 5.2, and 11.8 Pmol/mL, respectively. Sample recoveries for the three assays are between 80.6 and 101.7% Conclusion: Three
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Iatrogenic Vitamin D Toxicity in an Infant: Clinical Relevance of Vitamin D Metabolic Profiling

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Background: Public concern over vitamin D (vitD) deficiency has led to widespread use of over the counter (OTC) vitD supplements, containing up to 10,000 IU (400 IU=10 pg) of vitD. Overuse or misuse of such supplements can result in vitD toxicity. Infants are particularly vulnerable to toxicity associated with vitD overdose. Mutations in the CYP24A1 gene have been shown to cause reduced serum 24,25-dihydroxyvitamin D (25(OH)2D) to 25-hydroxyvitamin D 25(OH)D ratio (≥0.02), elevated serum 1,25-dihydroxyvitamin D (1,25(OH)2D), hypercalcemia, hypercalciuria and nephrocalcinosis. Additionally, studies in infants have shown that C3 epimer of 25(OH)D can contribute up to 9-61% of the total 25-OHD. Therefore, measurements of parathyroid hormone (PTH) and vitD metabolites 25(OH)D, 1,25(OH)2D, 3-Epi-25-hydroxyvitamin D (3EPI-25(OH)D) and 24,25(OH)2D is of clinical value for differentiating between genetic vs iatrogenic causes of hypercalcemia.

Case: A significantly underweight four month old female presented with a three day history of emesis, diarrhea, lethargy and dehydration. The medical work-up revealed hypercalcemia, hypercalciuria and nephrocalcinosis. She had been exclusively breastfed and had been given OTC vitD supplementation at a higher dose than recommended on the supplement’s label. 25(OH)D, 1,25(OH)2D, 3-EPI-25(OH)D and 24,25(OH)2D is of clinical value for differentiating between genetic vs iatrogenic causes of hypercalcemia.


Results: Nephrocalcinosis was confirmed by ultrasound studies. Serum calcium (SCa) was 18.7 mg/dL (ref range: 9.1-11 mg/dL) and PTH was < 6 fg/mL (ref range: 15-65 pg/mL) at presentation. Urine calcium was 157 mg/dL with a calcium to creatinine (Cr) ratio of 2618 mg/g of Cr (ref range < 2100 mg/g). The vitD content of the supplement was threefold higher (6000 IU of D3 over) than listed on the label (2000 IU). Combined with the gross overdosing, this was estimated to have resulted in a daily vitD dose of 50,000 IU for two months. The SCa gradually decreased upon calcitonin injection on day 3, but trended upward again (15.5, 13.3, 12.1, 10.4, 9.8, 10.7, 10.9, 10.3 and 11.6 mg/dL on days 1, 3, 5, 6, 11, 14, 20, 25 and 40 respectively). The 25(OH)D decreased slowly from 294 ng/mL on day 1 to 257, 227, 197, 189, 138, 124 and 84 ng/mL on days 3, 5, 7, 11, 14, 20, 25 and 40 respectively. The PTH decreased from 145 pg/mL at presentation to < 6 pg/mL at 25(OH)D levels were elevated. The ratio of 25(OH)D to 25(OH)D was 0.11-0.14 (ref range: 0.07-0.18). Combined with the gross overdosing, this was estimated to have resulted in a daily vitD dose of 50,000 IU for two months. The SCa gradually decreased upon calcitonin injection on day 3, but trended upward again (15.5, 13.3, 12.1, 10.4, 9.8, 10.7, 10.9, 10.3 and 11.6 mg/dL on days 1, 3, 5, 6, 11, 14, 20, 25 and 40 respectively). The 25(OH)D decreased slowly from 294 ng/mL on day 1 to 257, 227, 197, 189, 138, 124 and 84 ng/mL on days 3, 5, 7, 11, 14, 20, 25 and 40 respectively. The PTH decreased from 145 pg/mL at presentation to < 6 pg/mL at 25(OH)D levels were elevated. The ratio of 25(OH)D to 25(OH)D was 0.11-0.14 (ref range: 0.07-0.18).

Aim: To examine the natural history of insulin secretory dysfunction and insulin resistance during the development of diabetes and to examine the role of adipocyte mediators non-esterified fatty acids (NEFA) in development of type 2 DM.

Methods: The study was conducted on newly diagnosed, untreated hyperglycaemic patients attending our diabetic clinic. The study subjects were grouped as Group I: Healthy control (n = 56), Group II: Pre-diabetics (n=39), Group III: Diabetics (n=124). All subjects were evaluated for waist to hip ratio (W/H), body mass index (BMI), fasting blood glucose, insulin, HOMA-IR, HOMA-beta, NEFA and lipid profile. Statistical analysis was done using ANOVA and Multiple logistic regression analysis.

Results: The diabetic subjects had significantly raised W: H and BMI as compared to the pre-diabetics (0.88±0.06; 27.4±4.62) and controls (0.87±0.06; 24.2±4.34) with the F value 14.64 (p<0.001) and 5.98 (p=0.003) respectively for W: H and BMI. Age adjusted base line characteristics according to BMI and W: H quintiles for predicting risk of type 2 Diabetes showed significant trends across quintiles for total cholesterol (TC), triglycerides (TG), HOMA-IR and HOMA-beta. Similarly Age adjusted base line characteristics according to NEFA quintiles for predicting risk of type 2 Diabetes showed significant trends across quintiles for BMI, W: H, lipid profile, insulin, HOMA-IR and HOMA-beta. Finally Multiple logistic regression analysis in newly diagnosed type 2 diabetic subjects with family history (Negative vs Positive) as a dependent variable showed the strongest risk due to raised NEFA (OR 3.83), followed by HOMA-IR (OR 1.38), TCH (OR 1.35), WC (OR 1.7) and TG (OR 1.13).

Conclusion: We conclude that the insulin secretory rates and IR in pre-diabetics and newly diagnosed type 2 diabetes are associated with BMI and NEFA. W: H and NEFA can prove to be a strong predictor of type 2 DM even with a negative family history.
AMR self-management, solving linearity studies issues, ensuring additional safety and reliability of released results, and Laboratory’s Quality Control Improvement.

Table 1 -

<table>
<thead>
<tr>
<th>Assay</th>
<th>Assay Linearity Range</th>
<th>Obtained CV %</th>
<th>CV Lab max Target %</th>
<th>Obtained TE %</th>
<th>TE max Target %</th>
<th>Linear Regression</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BNP</td>
<td>85 - 2000 pg/mL</td>
<td>4.13</td>
<td>1.50</td>
<td>4.33</td>
<td>10.00</td>
<td>0.998 (± 0.874)</td>
<td>0.99739</td>
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<tr>
<td>FOL</td>
<td>1.25 - 50 ng/mL</td>
<td>2.80</td>
<td>1.50</td>
<td>3.80</td>
<td>10.00</td>
<td>0.998 ± (± 0.587)</td>
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<td>FER</td>
<td>0.95 - 1850 ng/mL</td>
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<td>3.90</td>
<td>16.00</td>
<td>0.999 ± (± 2.411)</td>
<td>0.99512</td>
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<td>BNP</td>
<td>2 - 5000 pg/mL</td>
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<td>1.50</td>
<td>2.50</td>
<td>22.43</td>
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<tr>
<td>FOL</td>
<td>0.75 - 75 ng/mL</td>
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<td>0.998 ± (± 1.233)</td>
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<tr>
<td>Pep C</td>
<td>0.35 - 24 ng/mL</td>
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<td>1.50</td>
<td>2.43</td>
<td>22.43</td>
<td>0.998 ± (± 2.420)</td>
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<td>INS</td>
<td>45 - 2000 ng/mL</td>
<td>4.13</td>
<td>1.50</td>
<td>4.13</td>
<td>10.00</td>
<td>0.998 (± 0.874)</td>
<td>0.99739</td>
</tr>
</tbody>
</table>

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Linearity study to define the Analytical Measurement Range (AMR) for immunoassays

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Background: Linearity studies are crucial to assure analytical range accuracy and to validate reagents performance in clinical laboratory. Due to difficulties to evaluate linearity results, a Brazilian laboratory implemented a new tool (Easy Linearity Curve, ELC). ELC established self-inspection program, immune-hormone analytical systems monitoring and quality control requirements accomplishment. We described linearity data for nine different immunoassays: BNP, Folates(FOL), Ferritin(FER), BNP, Cortisol (Cor), C-Peptide(Pep C) and Insulin(Ins).

Materials and methods: Samples were selected from laboratory routine, with concentrations within assay linearity range for each test, performed by chemiluminescence on Advia Centaur® XP (Siemens Healthcare Diagnostics). With concentrations within assay linearity range for each test, performed by

Results and Discussion: Tests showed satisfactory linearity results for different samples. They are summarized in the table below. The sample pools were not used, because all the results from this dilution test presented high percentage of recovery, higher than manufacturer’s references, due to a matrix effect (data not showed). The main difficulty in linearity studies is to obtain samples in the ideal analytical range. ELC uses an algorithm that suggests samples’ dilutions to contemplate the whole AMR for each parameter. In this study, for each analyte, five different samples were selected to simulate ideal concentrations covering the linear range.

Conclusion: Based on our results, we do not recommend the use of sample pools, instead we demonstrated feasibility of the AMR self-management, solving linearity studies issues, ensuring additional safety and reliability of released results, and Laboratory’s Quality Control Improvement.

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Analytical performance of the Insulin-like growth factor I (IGF-I) assay in two Immunoassays Systems that used different standardization procedures

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Background: Insulin-like growth factor I (IGF-I) is intended for use in the diagnosis and monitoring of children who have growth-related disorders and adults with acromegaly. Large variability exists among different IGF-I assays owing to differences in calibration, antibody specificity, isoform recognition. The World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) established criteria for standardization and evaluation of IGF-I assay systems (Recombinant IGF-I, coded 02/254) and for the content monitoring of therapeutic products.

Objective: This study intends to evaluate the analytical performance of the IGF-I assay on Siemens IMMULITE 2000 IGF-I (Recombinant IGF-I, coded 02/254) and Liaison Analyser IGF-I Diasorin (Recombinant IGF-I, coded 02/254).

Methods: We tested 59 patient’s serum samples from DASA, Rio de Janeiro, with concentrations within the range of 25 ng/mL to 1039 ng/mL, in both analytical platforms. The IMMULITE 2000 IGF-I is a 2-cycle, sequential immunometric assay without dilution range of 20-1600 ng/mL. In the assay procedure, prediluted patient sample is needed to reduce interference from binding proteins before analysis. The photon output is proportional to the concentration of the analyte. The Liaison Analyser IGF-I is a 1-cycle, immunometric assay with calibration range of IGF-I 10-1500 ng/mL.

Results: Comparison between IMMULITE 2000 and Liaison assays yielded a correlation coefficient of 0.97, with linear regression of x(IMMULITE 2000) = 0.792(Liaison) + 30.48 ng/mL. Moreover, means were 313 and 278 ng/mL for IMMULITE 2000 and Liaison Analyser, respectively. Test t-student was 1.049 (expected is 1.98) and estimated total error (TE) was 20.2% which is lower than the allowed TE (22.35%) in all levels of decision-making practice.

Conclusion: Preliminary assessment of these clinical evaluation results indicates that the IMMULITE 2000 IGF-I immunoassay is a precise method for measuring IGF-I in serum across a wide range of clinically relevant concentrations and shows good correlation to the Liaison IGF-I assay despite different standardization.

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Diiodothyropropionic acid interferes with TT3 and FT3 measurements on common Immunoassay Platforms for Thyroid Function Panel.

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Background: Monocarboxylate transporter 8 (MCT8) is a thyroid hormone-specific cell membrane transporter. MCT8 deficiency produces in young males an unusual pattern of thyroid hormone abnormality with elevated serum triiodothyronine (TT3) and causes severe neuropsychiatric defect. A thyroid hormone analogue, diiodothyropropionic acid (DITPA) was found to enter cells independently of MCT8.

Therefore, this compound was tested in children with MCT8 deficiency and found to normalize their thyroid function tests and improve their nutritional status. A problem in the follow up of DITPA treatment is the interference of DITPA in the routine laboratory measurement of TT3. This necessitates the measurement of DITPA by LC-MS/MS to correct for its interference in TT3 determination by immunoassay. The objective of this study was to evaluate the possible interference of DITPA on commercial thyroid assays available from four in-vitro diagnostic companies.

Method: Pooled human serum was collected and stock DITPA (1 mg/mL) was added to create a serum set containing 75-500 μg/dL DITPA. This sample set was then assayed for TT3, TT4, free T3, free T4, and TSH on the Roche Elecsys, Siemens IMMULITE, Siemens ADVIA Centaur, Siemens Dimension EXL, Siemens Dimension Rxl.
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Beckman Access, and Abbott Architect platforms, where available, respectively. All samples were analyzed in duplicate and the respective assay values in the DITPA-treated samples were expressed as the difference above the baseline sample. In addition, to investigate if the interference of DITPA could be overcome by excess TT3, serum samples at a constant concentration of 20 μg/dL DITPA were spiked with increasing amount of TT3 and measured on Roche Elecsys TT3 method. Results: At 75 μg/dL DITPA, the overestimation above the baseline endogenous values were: Elecsys: TT3, 193 ng/dL; FT3, >3000 pg/dL; Access: TT3, 745 ng/dL; FT3, 2405 pg/dL; IMMULITE: TT3, 146 ng/dL; Architect: TT3, 33 ng/dL, FT3, 96 pg/dL, respectively. Minimal interference was observed for ADVIA Centaur TT3 assay at all concentration of DITPA tested. TT4, FT4, and TSH tests were not affected by DITPA on all the immunoassay platforms tested. It is also interesting that with excess TT3, the DITPA interference on the Elecsys TT3 declined from 208% to 114% above baseline. Conclusion: DITPA significantly interferes with several commercial TT3 and FT3 assays with the exception of the Siemens ADVIA Centaur TT3 assay. This suggests that the TT3 antibody reagents used in the Elecsys, Access, Immulite and Architect cross-react significantly with DITPA. For patients undergoing treatment, the ADVIA Centaur TT3 assay is suitable for monitoring response to DITPA therapy.

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Gender Differences in the interactions between Adipokines and the Insulin-Like Growth Factor-I System in a Metabolically High Risk Population

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Background: Accumulating evidence indicate important roles for the insulin-like growth factor (IGF)/IGF-binding protein (IGFBP) system in metabolic homeostasis. Despite potential molecular mechanisms that link obesity and insulin resistance with the IGF-I system remain unknown due to conflicting reports in the literature. In this study, we test the hypothesis that gender differences could be responsible in part for the conflicting reports on the associations of some adipokines with the IGF system. Methods: Fasting adiponectin, resistin, leptin, leptin receptor (sOB-R), insulin, glucose, total IGF-I, IGFBP-3 and full lipid profile were determined in 590 (238M and 352F) first-degree relatives of patients with Type 2 Diabetes Mellitus. Sex hormone binding globulin (SHBG), androstenedione (AND), oestradiol (E2), testosterone (T), were also measured. Free androgen index (FAI), Free leptin index (FLI), bioavailable IGF-1 (BIGF1), insulin cell function (%IF), insulin sensitivity (%S) and insulin resistance (IR) (Homeostasis Model Assessment) were calculated. The data were analysed using simple and multivariate regression analyses.

Results: There are significant differences in mean (SEM) BIGF1 between males (87.6 (9.1)) and females (67.7 (4.6)). There were also significant gender differences in adiponectin, leptin, sOB-R, FLI, %S and IR. There were no gender differences in resistin and IGFBP3. Significant gender differences were found in the correlations of BIGF1. The following showed significant correlations with BIGF1 in females but not in males: adiponectin, sOB-R, FLI, IR, %S and IR, waist circumference, BMI, Apo B, total cholesterol, triglycerides and LDL-cholesterol. Males and females showed similar correlations of all other variables with BIGF1. Correlations with sex hormones (E2, T, SHBG, FAI) were not significant in males and females. Multivariate linear regression analysis showed that age, BMI, WC, adiponectin, FLI were significant determinants of BIGF1 in females but not in males. Age was the only significant determinant of BIGF1 in males.

Conclusions: There are significant gender differences in the metabolic interaction between adipokines and the IGF-I system. Despite the putative links with obesity, sex steroids do not play a role in the gender differences.

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LC-MS/MS detection of increased Androstenedione levels in patients receiving Danazol therapy

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Background: Danazol is a 17-ethyl testosterone derivative. Danazol has long been used in the management of endometriosis, however its reported immunomodulatory effects such as reducing interleukin-1 and TNF-a have led to the use of danazol in management of immune related conditions such as aplastic anemia. The side effects associated with danazol are largely due to its androgenic effects. Danazol has been reported to act as an interference in the immunoassay measurement of various androgens (including androstenedione), resulting in falsely elevated values for these hormones. As a consequence measurement of these hormones in patients receiving danazol is best performed by liquid chromatography tandem mass spectrometry (LC-MS/MS). Here we report eight cases of significantly elevated androstenedione (AND) levels following LC-MS/MS measurement in patients receiving danazol for aplastic anemia.

Method: LC-MS/MS measurement: AND is measured as part of a steroid hormone panel. Samples were prepared as previously published method (Guo et al. Simultaneous determination of 12 steroids by isotope dilution liquid chromatography-mass spectrometry, J Chromatogr A 2006;1099:23-62 and Mendum et al. Dr et al Clin Chem 2011 Abstract E-57, pA212). An Agilent 6400 triple quadrupole mass spectrometer (Agilent, USA) equipped with an atmospheric pressure photoionization was used, employing isotope dilution with deuterium labeled internal standard for each analyte. Quantitation by multiple reaction monitoring (MRM) was performed in the positive ion mode. The quantifier MRM transition for androstenedione used was 287.2→97.1 and the qualifier MRM of 287.2→109 was used to confirm. The ratio of response of the two MRMs used, ranged from 56-58%. Retention times (RT) for AND were 6.540–6.556 minutes for patient samples and 6.529–6.554 minutes for the accompanying internal standard (IS). The ratio of androstenedione RT to internal standard RT for each specimen run was 1.00, thus confirming the identity of the peak.

Results: A total of 8 adult patients (female n=5; male n=3) were identified with increased AND values at either 6 or 12 month follow-up post danazol initiation. Baseline AND values for the female patients ranged from 38 - 176 ng/dL (reference interval 17-175) and at 6 or 12 month follow up values increased markedly and ranged from 8128-33703 ng/dL. For the male patients baseline AND values ranged from 105-240 ng/dL (reference interval 25-125) and at follow up values increased to range from 5609 to 17325 ng/dL. Similar increases were not observed for the other androgens measured. Of interest two of the three males had elevated LC-MS/MS androstenedione levels above the reference interval on follow-up while all the female patients had estradiol concentrations that remained within the appropriate estradiol reference interval during therapy. Whilst on therapy patients responded well and side effects of therapy were reported to be minimal.

Conclusion: One of the important advantages of MS analysis is the greater specificity of over immunoassay based testing. In the above described cases use of two MRM transitions (quantifier and qualifier) enabled the laboratory to confirm the presence of elevated AND and exclude the likelihood of an interference.

Evaluation of the effect of elevated Fetal Hemoglobin (HbF) on three HbA1c Assays Methods in Marshfield Clinic system.

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Background: Accurate measurement of HbA1c is crucial for decision making in diabetic control and diagnosis. Elevated levels of HbF are reported to falsely decrease the HbA1c results. There are many clinical conditions presenting with elevated HbF and prevalence of elevated HbF can be as high as 7 to 8% in a diabetic population. Clinicians may be unaware of potential interference with HbA1c results. At a glycomic control target of 6.5% the critical difference between two results within a glycemic control target of 6.5% the critical difference between two results within a patient should not exceed ~0.4%. It is therefore, crucial that laboratories are aware, to what extent HbF interference affects HbA1c results. Clinicians can then make informed decisions if HbA1c results interfere from elevated HbF levels on a particular method that could adversely affect HbA1c results. Clinicians can then make informed decisions if HbA1c results appear discrepant related to patient history and glucose hemostasis.
markers (BTMs) are renally cleared; thus, in CKD they may be non-specific. Invasive turnover determination in CKD is controversial because most bone turnover is currently determined by tetracycline double-labeled transiliac crest bone biopsy. Estimated glomerular filtration rate (eGFR) was by MDRD formula. Total serum PINP (mono- and multi-meric) was measured by Electrochemiluminescence immunoassay (Elecys 2010 analyzer, Roche Diagnostics, Indianapolis, IN). Intra- and inter-assay precision were 1.1% and 5.5% respectively (Reference range = 20-100 μg/L). BFR (μm²/μm²/day) was determined histomorphometrically in trabecular, endocortical and intra-cortical bone from biopsy using American Society for Bone and Mineral Research (ASBMR) criteria. Data are presented as mean±SD. PINP and BFR were log transformed prior to analyses and relationships were determined by Pearson correlations.

Results: Five patients were on hemodialysis and mean eGFR in pre-dialysis patients was 36±17 ml/min. Mean BFR at trabecular, endocortical and intracortical regions were 0.018±0.031, 0.019±0.035 and 0.030±0.038 respectively and there were no significant differences in BFR between pre-dialysis and hemodialysis patients. Mean PINP for the total, pre-dialysis and hemodialysis cohorts were 332±585 μg/L, 98±51 μg/L and 1125±387 μg/L respectively. PINP levels were significantly greater in hemodialysis compared to pre-dialysis (p=0.004) and there were no significant relationships between PINP and eGFR among pre-dialysis patients. There were significant, moderate and direct associations between PINP and BFR in the three envelopes (R² 0.41, 0.34 and 0.34, all p<0.05 for trabecular, endocortical and intracortical bone, respectively).

Conclusion: These data suggest that measurement of total serum PINP by the Elecsys assay correlates well with BFR in CKD. Larger studies are needed in CKD populations to validate these data, and to determine whether PINP predicts future fracture and can be used to guide treatment to protect against bone loss and fracture.

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The contribution of angiotensin II-dependent oxidative stress to megalin expression in the renal cortex during the normoalbuminuric stage of diabetes mellitus in the rat.

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Background: Renal albuminuria can result from impaired albumin handling by the glomerulus or the renal tubules. Megalin plays a critical role in proximal tubular albumin reabsorption, and altered megalin expression or function can contribute to renal tubular albuminuria. Elevated glucose reabsorption during acute hyperglycemia results in impaired tubular handling of protein and proximal tubular damage (Enzyme Protein 8:243-50, 1994-1995). Moreover, renal albuminuria during diabetes mellitus (DM) is associated with decreased megalin expression in the proximal tubule (Diabetes 56:380-388, 2007). During the early stage of diabetes mellitus (DM) in rats, prior to development of albuminuria, the renal cortex exhibits oxidative stress that can be suppressed by renin-angiotensin system (RAS) inhibition (Clin Sci 24:543-52, 2013); however, it is not known whether these events arising during the normoalbuminuric stage of DM influence megalin expression and protein excretion.

Objective: The goal of this study was to evaluate impact of oxidative stress suppression, achieved by angiotensin II receptor blocker (ARB) treatment, on proximal tubular megalin expression during the normoalbuminuric stage of DM in rats.

Methods: Four groups of rats were examined: 1) STZ group (n=5): rats studied 2 weeks after induction of DM by streptozotocin injection (STZ, 65 mg/kg, i.p.), 2) Sham group (n=5): rats receiving the STZ vehicle, 3) STZ-TLM group (n=4): STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham-TLM group (n=4): TLM-treated Sham rats. In each rat, blood glucose, blood pressure, glomerular filtration rate (GFR) were measured, as well as urinary albumin levels and activity of N-acetyl-β-D-glucosaminidase (NAG; a proximal tubule-derived enzyme) in urine. Further, we measured renal cortical 3-nitrotyrosine (3-NT) production (oxidative stress marker) by HPLC and megalin expression (Western blot analysis).

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Procollagen of type-I N-terminal propeptide levels by Elecsys assay correlates with bone formation rate in Chronic Kidney Disease

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Background: Renal osteodystrophy is a common metabolic bone disorder due to diabetic kidney disease (CKD). The severity of this disease is proportional to the quantity of the color of the reagent pad. The instrument measures the color change of the reagent pad on a scale of 0 to 4000.

Methods: Recent urine microalbuminuria of diabetic patients were determined by two methods: 1. Semi-quantitative: Colorimetric method using the strip H13 in DIRUY H-800 PLUS (RAI-8). The content of microalbuminuria is inversely proportional to the quantity of the color of the reagent pad. The instrument measures the color change of the reagent pad on a scale of 0 to 4000. 2. Quantitative: microalbuminuria was measured by immunoturbidimetry in COBAS C311 (ROCHE DIAGNOSTIC®). Patients were classified into two groups according to the quantification of microalbuminuria: positive (microalbuminuria > 20 mg/L) and negative (microalbuminuria < 20 mg/L). Statistical analysis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

Results: We analyzed 469 diabetic patients between 27 and 85 y.o. (mean age = 56.3), 82 patients (17.5%) had a positive microalbuminuria and 387 patients (82.5%) were negative. The AUC was 0.985 (p < 0.0001). With a cut-off color scale less than 1305 determined by the test strip, we obtained a sensitivity of 100% and a specificity of 86.3%. With these results, it would only be necessary the quantification by immunoturbidimetry the samples with a value lower than 1305. In this case it would only be necessary to measure microalbuminuria in 334 samples of the 469 studied, getting a saving of 71%.

Conclusions: The semi-quantitative method by test strip, can be used as screening for microalbuminuria in diabetic patients with a sensitivity of 100%. Microalbuminuria would only be measured in samples with positive test strip.
Results: Blood glucose levels were higher in STZ and STZ+TLM groups than in Sham and Sham+TLM groups (P<0.05), confirming development of DM; however, blood pressure and urine albumin level did not differ among groups. GFR and urinary NAG activity (an index of proximal tubule damage) were increased in the STZ group compared with Sham (each P<0.05), and these changes were prevented by TLM-treatment (each P<0.05 STZ vs. STZ+TLM). Renal cortical NT-protein expression in the STZ group was 70% greater than in the Sham group (Sham, 34±1.0 pmoi/mg protein; STZ, 58±4.2 pmoi/mg protein; P<0.05 vs. Sham); however, this phenomenon was completely suppressed by TLM treatment (STZ+TLM, 35±1.5 pmoi/mg protein; P<0.05 vs. STZ). Renal cortical megalin expression was elevated in the STZ group (30±8% of Sham; P<0.05); however, the enhanced expression of megalin in the STZ group was not evident in the STZ+TLM group (4%±61% of Sham; P<0.05 STZ vs. STZ+TLM).

Conclusions: These observations demonstrate that increased renal cortical megalin expression accompanies oxidative stress during the early stage of DM, prior to development of albuminuria. The ability of ARB treatment to prevent the DM-induced elevation of megalin implicates the renin-angiotensin system in this phenomenon, perhaps through an oxidative stress-dependent mechanism.

A-205

Analytical validation of the new Roche Thyroglobulin II electrochemiluminescent immunoassay.

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Background: Thyroglobulin (Tg) is a serum marker of thyroid cancer. It is thus critical to measure it accurately in the low range of 0.1 to 1.0 ng/mL level. Unfortunately, this is not possible with most commercial kits, including the traditional Roche electrochemiluminescent Tg assay. One exception is the Beckman-Coulter Tg chemiluminescent assay (Brea, USA), which has a limit of quantitation (LOQ) of 0.1 ng/mL and, for this reason, is considered by many the reference method. In this study, we evaluated the analytical specifications of the new Roche Tg II electrochemiluminescent immunoassay (Mannheim, Germany).

Methods: All serum samples selected for this protocol were routine clinical specimens previously assayed for Tg in our lab using the Beckman-Coulter Access II® assay. They were all run within one week in Roche E170 Modular Analytics® using the new Tg II assay. This is an electrochemiluminescent immunometric assay using 2 monoclonal antibodies to form a sandwich complex with Tg. Three samples with low, normal or high Tg levels were run in Modular E170, all in duplicate in the morning and afternoon for 5 days, and then used to calculate intra and interassay variation and LOQ (lowest concentration with CV ≤ 20%). Linearity and analytical measuring range (AMR) were evaluated in Modular E170 by mixing a low and high Tg sample in different proportions. Correlation studies were performed in 46 samples assayed in both Access II and Modular E170. Results were analyzed with EP Explorer 11.0 software (Data Innovations, South Burlington, USA).

Results: Intra and interassay CV were 2.3% and 5.1% at 0.28 ng/mL; 1.5% and 2.7% at 2.2 ng/mL; and 0.7% and 2.3% at 45.9 ng/mL, respectively. LOD was set at ≤ 0.2 ng/mL, based on the low CV found at this level. The assay was linear at 0.21-493.3 ng/mL, which was defined as the AMR. Modular E170 results were compared with Access II using Deming regression over a range of 0.20 to 34.4 ng/mL. The correlation coefficient was 0.96, average error index 1.50 (range -3.71 to 6.41), slope 1.47, intercept -0.46 and standard error of estimate 3.17. The methods were not considered equivalent within allowable total error of 21.9% (biologic variation database, Ricco C 2012).

Conclusion: The new Roche Tg II assay exhibited an excellent precision and linearity down to 0.2 ng/mL. Although results correlated well with the Beckman-Coulter Tg assay, they were not considered equivalent to this reference method.

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Prediabetic Importance of Serum Zinc Alpha Glycoprotein and Ghrelin Levels in Subjects Classified According to Oral Glucose Loading Test and Fasting Glucose Levels

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Background: Increasing evidence suggests that the postprandial state and fasting hyperglycemia are a contributing factor to the development of Diabetes Mellitus. It has been recently suggested that an adipokine, zinc-a2-glycoprotein (ZAG), may also have a protective role in the prevention of obesity and its associated disorders. ZAG has been proposed to play a role in the pathogenesis of insulin resistance and suspected to be related with Type 2 Diabetes. Ghrelin a peptide hormone secreted mainly by the stomach, increases appetite and food intake. It has been suggested that Ghrelin hormone plays role in insulin secretion and glucose metabolism.

In the present study we determined serum ZAG and Ghrelin levels, and evaluated whether the relationship between serum ZAG and ghrelin levels in prediabetic stages. Methods: Subjects were categorized according to WHO criteria as Controls (n=23; women:13, men:10, mean age:55.6 ± 7.7 years ), Impaired Fasting Glucose (IFG; women:29, men:23, mean age: 55.1 ± 7.0 years), Impaired Glucose Tolerance (IGT; women:26, men: 20,mean age: 59.1 ± 8.4 years) and Diabetic Glucose Tolerance (DGT; women:15,men: 15, mean age 59.9 ± 11.1 years) in our study. There was no any difference in Body Mass Index and plasma lipids levels (total cholesterol, triglyceride, HDL and LDL, cholesterol) between groups. Subject’s patients did not use any medication or vitamin pills. Baseline serum ZAG and Ghrelin levels were determined by ELISA. Serum insulin levels were determined by chemiluminesanss assay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated.

Results: Serum ZAG levels in Control group were found to be significantly higher than in DGT, IGT and IFG groups (p<0.005, p=0.001 and p<0.001 respectively). IFG group have significantly lower serum ZAG levels than both DGT and IGT groups (p<0.005 and p<0.001).

Conclusion: Serum levels of ZAG and Ghrelin may be possible marker for the 2-hours post challenged glucose levels. The present works was supported by the Research Fund of Istanbul University. Project No: 29822

A-207

Angiotensin II-dependent oxidative stress and increased hypoxia-inducible factor-1α expression in the renal cortex during the normoalbuminuric stage of diabetic mellitus in the rat.

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Background: Hypoxia-inducible factor-1 (HIF-1) is composed of α and β subunits, with α considered to be a master regulator of the hypoxic response. HIF-1α and oxidative stress are associate with the progression of diabetic nephropathy (Clin Exp Pharmacol Physiol 33:997-1001, 2006). We previously reported that renal cortical oxidant production is already increased during the early stage of diabetes mellitus (DM), prior to development of albuminuria, and that renin-angiotensin system (RAS) inhibition suppresses development of renal oxidative stress under these conditions (Clin Sci 124:543-52,2013). However, the relationship between oxidative stress and the hypoxic response in the kidney during the normoalbuminuric stage of DM has not been established.

Objective: The goal of this study was to determine the effect of oxidative stress suppression by RAS inhibition (treatment with an angiotensin II receptor blocker; ARB) on HIF-1α expression in the renal cortex during the normoalbuminuric stage of diabetic mellitus in the rat.

Methods: Four groups of rats were examined: 1) STZ group (n=5): rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg, i.p.), 2) Sham group (n=5): rats receiving the STZ vehicle, 3) STZ+TLM group (n=5): STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day in chow for 2 wks), and 4) Sham+TLM group (n=4): TLM-treated Sham rats. In each rat, blood glucose, blood pressure and glomerular filtration rate (GFR) were measured. Production of 3-nitrotyrosine (3-NT; an oxidative stress marker) in the renal cortex was measured by HPLC, and HIF-1α expression was quantified by the Western blot analysis.

Results: Blood glucose levels were significantly higher in STZ rats than in Sham rats, and was unaffected by TLM (similar to other treatments that suppress the RAS). Blood pressure did not differ among groups. Compared with the Sham group, GFR...
was increased in the STZ group ($P<0.05$), and this was prevented by TLM treatment ($P=0.05$ STZ vs. STZ+TLM). Renal cortical 3-NT production in the STZ group was 70% greater than in the Sham group (Sham, 35.2±3.4 pmol/mg protein; STZ, 59.6±1.4 pmol/mg protein; $P<0.05$ vs. Sham); however, this phenomenon was completely suppressed by TLM treatment (STZ+TLM, 37.3±3.5 pmol/mg protein; $P<0.05$ vs. STZ). The STZ group also displayed an increase in renal cortical HIF-1α expression (257±26% of Sham; $P=0.05$); however, the DM-induced increase in HIF-1α expression was not evident in the STZ+TLM group (157±11% of Sham; $P=0.05$ STZ vs. STZ+TLM).

Conclusions: An increase in renal HIF-1α expression accompanies oxidative stress during the normoalbuminuric stage of DM in the rat, and both of these phenomena are prevented by ARBs. These observations indicate that the hypoxic response arises in the renal cortex early during the course of DM, and that this occurs either directly or indirectly under the influence of the RAS, possibly secondary to the presence of oxidative stress.

**A-208**

Evaluation of TSH Levels in Rio de Janeiro State/Brazil Neonatal Screening.

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Background: Congenital Hypothyroidism (CH) is the most common congenital endocrine disorder and it is a leading preventable mental retardation. Its incidence ranges from 1:2000 to 1:4000 live births in iodine-sufficient countries. In Brazil, the screening for CH is mandatory by law and usually done by TSH determination on dried blood spot on filter paper samples collected by heel puncture. Diagnostic confirmation is required dosing TSH and free T4 in serum. The objective of this study was to evaluate the distribution of TSH levels in newborns’ blood samples from Rio de Janeiro state and the frequency of CH confirmed patients. We also compared our results to those described by the manufacturer.

Methods: We evaluated 18,609 dried blood spots on filter paper samples for TSH of newborns from Rio de Janeiro state over one year period (2013). The range of age was 3 to 30 days of life. We used an automated immunofluorimetric system, GSP Neonatal iTSH kit (Wallace Oy, Turku, Finland). The cutoff value for TSH was 10.0 mU/L, children with levels above these limits were recalled for confirmation with serum TSH and FT4.

Results: The most of TSH levels, 85.3%, were less than 2.0 mU/L. The percentiles 95 and 99 were 3.0 and 4.7 mU/L, respectively. Comparing to the percentiles described by the manufacturer, 7.9 and 10.7mU/L, our results were much lower. We found 9 samples (0.05%) above the TSH cutoff. All these patients underwent measurement of serum TSH and FT4. Congenital Hypothyroidism was confirmed in 7 (1:2658) patients. Their initial TSH filter paper level ranged from 12.8 to 279.0 mU/L, with mean 115.0 mU/L and median 42.8 mU/L.

Conclusion: Our data are indicative that, in this Brazilian population, the distribution of TSH levels in newborn’s blood filter samples were lower to those presented by the manufacturer, based on European individuals studies. This reinforces the need for each laboratory to evaluate the TSH levels in its specific population. A Brazilian laboratory implemented an Easy Linearity Assay Assay Linearity study of thyroid assays assuring the quality control requirements.

**A-209**

Linearity study of thyroid assays assuring the quality control requirements

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Background: The ability to quantitate circulating levels of thyroid hormones is important in evaluating thyroid function. It is especially useful in the differential diagnosis of primary (thyroid) from secondary (pituitary) and tertiary (hypothalamic) hypothyroidism. To certify released results accuracy, it is important to ensure the linearity of the tests. A Brazilian laboratory implemented an Easy Linearity Curve(ELC) tool to monitor the immune-hormone analytical systems, establishing a self-inspection program to verify the efficiency and accuracy of procedures and results. The use of a tool that checks assay linearity provides additional safety and reliability of the results. This study aims to use the statistic tool to monitor the AMR of TSH, FT4, FT3 and T3.

Materials and methods: We selected samples from the laboratory routine, with concentrations within assay linearity range for each test. Samples were tested in Advia Centaur® XP (Siemens Healthcare Diagnostics) using a chemiluminescent method and analyzed with the ELC tool.

**A-210**

A REVIEW OF 312 GROWTH HORMONE STIMULATION TESTS PERFORMED AT A REFERENCE LABORATORY (DASA-RJ) IN RIO DE JANEIRO - BRAZIL

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Background: There is still no consensus on the stimulation test considered the “gold standard” for the diagnosis of GH deficiency. The optimal criteria for a definitive test of growth hormone function are not met by any single stimulus. Lack of standardization of GH response to each type of stimulus, poor reproducibility and lack of correlation between the response to the test and “growth” are some of the various limitations of these tests. The aim of our study was to examine the main tests of GH stimulus applied in our environment so as the response to these tests.

Materials and Methods: We did a retrospective review of 312 patients submitted to GH stimulation test in a period of 12 months. A test was considered responsive when peak GH >5ng/mL.

Results: GH stimulus with Clonidine was the most requested test. The mean age of our patients was 10.2 years, and male:female ratio was 2:4:1. Most of patients were therefore male. Interestingly, however, among patients submitted do the Glucagon simulation test, the majority were female. The greatest GH peak was seen with Glucagon stimulus. No significant complications were observed with the applied tests.

<table>
<thead>
<tr>
<th>STIMULUS</th>
<th>N (%)</th>
<th>Mean AGE (years)</th>
<th>GENDER (M/F ratio)</th>
<th>RESPONSIVI- NITY (%)</th>
<th>mean GH peak (ng/mL)</th>
<th>GH peak (time)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLONIDINE</td>
<td>192 (63.7%)</td>
<td>10.1</td>
<td>2.9</td>
<td>73</td>
<td>9.5</td>
<td>60' and 90'</td>
</tr>
<tr>
<td>INSULIN</td>
<td>35 (11.6%)</td>
<td>11.6</td>
<td>2.1</td>
<td>50</td>
<td>7.0</td>
<td>60'</td>
</tr>
<tr>
<td>GLUCAGON</td>
<td>28 (8.8%)</td>
<td>6.5</td>
<td>2.2</td>
<td>10</td>
<td>10.7</td>
<td>120'</td>
</tr>
</tbody>
</table>

Conclusions: The high number of tested patients so as the high rate of GH response to Clonidine suggests that this is the preferred screening test by test prescribers. On the other hand, the Insulin simulation seems to be preferably reserved to confirm the diagnosis of GH deficiency, since the number of patients submitted to these test was significantly smaller and the percentage of responders was only 50%. The use of Insulin as a second-line test in the investigation of GH deficiency is easily explained by the justified fear of potential complications associated with this test. Moreover, Glucagon stimulation is the preferred screening test in children under 6 years.

**A-211**

Strategy to Improve Diabetes diagnosis in Primary Care: Preliminary Results and Evaluation.

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BACKGROUND: With the introduction of HbA1c as a tool to diagnose diabetes, a strategy was designed, established and evaluated in consensus with general...
practitioners (GP) to detect diabetic patients through an opportunistic study to improve HbA1c requesting, and to ascertain if previous HbA1c demand was appropriate to detect diabetes.

METHODS: The laboratory decided to approach the GPs to design a strategy that would improve the diabetes diagnosis efficiency: Laboratory Information System (LIS) automatically would add HbA1c to every sample from primary care patients older than 45 years, without an HbA1c request in the previous three years and glucose results between 100 and 126 mg/dl. If results were above 6.4%, LIS recommended a second request in 3-6 month period. In a last meeting the strategy was approved, established March 1st 2013 and evaluated after a 6 month period. HbA1c was measured using a Variant™ II Turbo Hemoglobin Testing System (Bio-Rad Laboratories, Madrid, Spain).

RESULTS: 412 HbA1c were added automatically, causing 39 HbA1c values above 6.4%. After medical record review, 6 HbA1c results above 6.4% were justificated. To eleven patients a second HbA1c was requested, being 4 diagnosed as diabetic patients (HbA1c >6.4%) and in seven patients the second HbA1c result did not confirm diabetes. Despite their abnormal HbA1c results, until now, to 17 patients has not been requested a second HbA1c to confirm/discard the illness. Results are showed in figure. The cost of adding the 412 HbA1c was 535.6 US dollars. At this moment, each of the seven diabetes diagnosed represented a cost of 76.5 US dollars.

CONCLUSION: Our proposed opportunistic screening to discover diabetes seems cost-effective. Hba1c was previously under requested.

A-212
Measurement of serum testosterone, androstenedione and dehydroepiandrosterone (DHEA) levels using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS)

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Background: The adrenal and gonadal androgens testosterone, androstenedione and dehydroepiandrosterone (DHEA) play an important role in sexual development and fertility as well as in several other processes.

Methods: We developed a method to assess serum testosterone, androstenedione and DHEA levels in one run using Isotope-Dilution Liquid-Chromatography Tandem Mass Spectrometry (ID-LC-MS/MS). Sample preparation consisted of addition of internal standards (13C5-testosterone, 13C1-androstenedione and 14H1-DHEA) and a liquid-liquid extraction using hexane-ether. The samples were analyzed on an Acquity 2D UPLC system (Waters), equipped with a C4 column (Waters) and a Kinetex Fluorophenyl column (Phenomenex), and a Xevo TQ-S tandem mass spectrometer (Waters). The three analytes were baseline separated in a total run time of 9 minutes. The calibration curves ranged from 0.10 to 26 nmol/L for testosterone and androstenedione, and from 0.96 to 78 nmol/L for DHEA.

Results: The intra-assay CVs were <4.0%, <4.6% and <7.0% for testosterone, androstenedione and DHEA, respectively. The inter-assay CVs were <6% for testosterone and <8% for androstenedione and DHEA. A lower concentration inter-assay CVs were 15.0%, 7.0% and 9.3% for testosterone (0.08 nmol/L), androstenedione (0.47 nmol/L) and DHEA (1.18 nmol/L), respectively. Recoveries of spiked analytes were 101-107%, 99-106% and 92-104% for testosterone, androstenedione and DHEA, respectively. Linearity was shown in dilution series (mean R2 was >0.999 for all analytes). This method tested negative for interference from DHEA-sulphate, estrone, 17β-estradiol, androstenedione, 17-progesterone, dehydrotestosterone, epi-testosterone, cortisol and cortisone and did not show ion suppression. The method was shown to be suitable for serum as well as EDTA and heparin plasma.

The present testosterone method compared well (y = 1.00 x + 0.035 nmol/L; r = 0.9982) to another ID-LC-MS/MS method for testosterone concordant with a published reference method (Bui et al. 2013). In the near future, the present method will also be compared to another LC-MS/MS method for androstenedione and DHEA.

Conclusion: We developed a sensitive and accurate method to measure serum testosterone, androstenedione and DHEA levels in one run.

A-213
Glucagon Quantification: Comparison of Radioimmunoassay and Sandwich ELISA methods

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Background: Accurate and robust measurement of glucagon is important in understanding glucagon’s role in glucose metabolism and homeostasis as well as its role in the pathology of type 2 Diabetes and other metabolic diseases. It is also commonly used in clinical studies as a surrogate marker of drug efficacy. Competitive RIA methods, like the ALPCO Glucagon RIA (A), have been the gold standard for measuring glucagon but can be limiting due to the short shelf-life, long ordering lead times, long assay times, and large sample volume required (≥1 mL). The objective of this study is to evaluate the performance of the R&D Systems Glucagon Quantikine ELISA (R) and the Merckodia Glucagon ELISA (M) relative to the FDA cleared ALPCO Glucagon RIA.

Methods: All three assays were validated for precision, linearity, recovery, sensitivity, and normal glucagon ranges using fasting plasma samples collected in either K2EDTA or P300 tubes, frozen and stored at -70°C. A set of fasting and non-fasting P300 plasma samples were also used to directly compare glucagon results of all three assays.

Results: Precision for all three methods was acceptable with intra- and inter-assay precision being less than 9% for all three assays and inter-assay precision being 3.8 - 11.9% (A), 4.9 - 9.2% (R), and 4.3-8.1% (M). Dilutional linearity was acceptable up to 40-fold (A), 16-fold (R), and 16,000-fold (M) dilutions for the three assays. The Merckodia ELISA was the most sensitive with a lower limit of quantitation (where the % CV is equal to 20%) of 1.5 pmol/L (M) versus 8.6 (A) and 9 pmol/L (R) for the two other kits. The most striking difference in the three assays was in the glucagon values observed in apparently healthy donors. The mean glucagon values for normal samples analyzed using the ALPCO RIA method were much higher (39.3 pmol/L) than the R&D systems (28.8 pmol/L) and Merckodia (9.1 pmol/L). Although the R&D systems ELISA had mean normal glucagon values that were more in line with those obtained for the ALPCO RIA, Deming Regression Analysis using the same set of P800 plasma samples yielded a correlation coefficient of 0.6445 and slope of 2.174, while the Merckodia ELISA had a correlation of 0.9903 and a slope of 0.66 when compared with the ALPCO RIA. The majority of the twenty samples analyzed with the R&D systems ELISA yielded glucagon values 30-50% lower than the ALPCO RIA method, but there were three samples that had glucagon values that were higher in the R&D Systems ELISA than the ALPCO RIA which resulted in poor correlation. The Merckodia ELISA glucagon values were consistently lower than the ALPCO RIA values with greater biases observed for samples less than 10.0 pmol/L. Potential cross-reactivity with other glucagon-related molecules is speculated and currently being investigated as it may account for some of the differences observed between the three assays.

Conclusion: The Merckodia Glucagon ELISA may be a suitable alternative to the ALPCO Glucagon RIA method especially when sample volumes are limiting and better sensitivity is required.

A-214
Associations of Leukocyte Telomere Length with Cardiometabolic Risk Factors and Inflammatory Biomarkers in Non-diabetes Patients

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Background: Telomeres are TTAGGG sequences at the end of chromosomes necessary for and chromosomal integrity which upon reaching critical length, cell become senescent or otherwise dysfunctional. However, telomerase a reverse
Conclusions: T2DM [OR=0.8, p=0.008]. Higher hTERT levels though were associated with lower risk of inflammatory process, could play a role in mediating telomere shortening. Since, obesity and T2DM are increasing at epidemic pace in Kuwait; telomere attrition & telomerase levels could be potential cardio-metabolic risk markers of obesity and T2DM.

Methods: Indices of obesity (Body Mass Index [BMI], Waist Circumference [WC], Waist to height Ratio [WHHR], glycated Hemoglobin [HbA1c%], lipid profile, fasting glucose, serum human Telomerase Reverse Transcriptase [hTERT], total adiponectin, Insulin, Myeloperoxidase [MPO], Malondialdehyde [MDA], Total Oxidative stress status [TOS] and Leukocyte Telomere Length [LTL] were measured in 225 T2DM patients and 245 age and sex matched controls. Insulin resistance [IR] was estimated using Homeostasis Model Assessment [HOMA] calculator.

Results: T2DM patients had significantly (p<0.001) lower LTL compared to controls [(Mean±SD:2.2±1.02) vs. (Mean±SD:4.1±1.01)] respectively. Levels of hTERT were higher in controls compared to T2DM patients [(Mean±SD: 32.9±8.9 ng/mL) vs. (Mean±SD: 21.6±4.7 ng/mL)]. Spearman’s rank correlation coefficients showed that LTL correlated negatively with age [r=−0.2, p=0.009], BMI [r=−0.3, p=0.006], WC [r=−0.3, p=0.001], and Insulin [r=−0.2, p=0.03]. The significance of these correlations disappeared after adjusting BMI but not age and/or sex. Additionally, LTL correlated negatively and strongly with WHR [r=−0.5, p=0.004], and HbA1c% [r=−0.6, p=0.003]. These significant correlations were not affected by BMI, age or sex. Multiple regression analysis showed that LTL negatively associated with BMI [β=−0.7, p=0.005], WC [β=−5.7, p=0.004], HOMA-IR [β=−1.1, p=0.003], MPO [β=−0.6, p=0.001], MDA [β=−0.1, p=0.04], TOSβ=−2.2, p=0.001]. hTERT showed similar trends in relation to BMI [β=−0.2, p=0.004], WC [β=−1.4, p=0.006], HOMA-IR [β=−1.3, p=0.007], MPO [β=−0.6, p=0.001], MDA [β=−0.4, p=0.002], TOSβ=−0.3, p=0.007]. On the other hand, LTL and hTERT were associated significantly and positively associated with adiponectin [β=3.1, p=0.002; β=1.5, p=0.003] respectively. Using binary logistic regression analysis, higher BMI was associated with higher risk of telomeres’ shortening [OR=2.4, p=0.008]. Higher WC and WHR were associated with higher risk of telomere shortening [OR=2.4, p=0.001]; (OR=1.9, p=0.002)] respectively. Other obesity related factors such as IR, hyper-insulinemia and hyper-triglyceridemia [OR=7.7, p=0.001], (OR=1.2, p=0.001), (OR=1.3, p=0.01)] were also associated with higher risk of short telomeres. Higher levels of adiponectin were associated with lower risk of telomere shortening [OR=-0.7, p=0.004]. Additionally shorter telomere length were associated significantly with higher risk of T2DM [OR=7.5, p=0.003]. Higher hTERT levels though were associated with lower risk of T2DM [OR=0.8, p=0.008].

Conclusions: Our results demonstrate the link between telomere biology, cardiometabolic risk factors, and T2DM in the Kuwaiti population which has not been studied before. Metabolic changes such as the dys-regulation of adipokines, dys-lipidemia, hyper-insulinemia, IR and obesity associated inflammatory process, could play a role in mediating telomere shortening. Since, obesity and T2DM are increasing at epidemic pace in Kuwait; telomere attrition & telomerase levels could be potential cardio-metabolic risk markers of obesity and T2DM.

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Assocations of Common TERC Single Nucleotide Polymorphisms with Telomere Length, Human Telomerase Reverse Transcriptase and Obesity Related Factors

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Background: Quantitative trait locus studies have mapped putative loci that probably are involved in the regulation of leukocyte telomere length [LTL] to human chromosomes 3p26.1, 10q26.13, 12q12.22 and 14q23.2. The strongest associations with LTL were reported for SNP rs12693604 and rs16847897 near TERC on 3q26. It is unclear though whether this locus identified in Europeans, American, and Chinese exerts a similar effect on LTL in other populations. Additionally, the effect of such SNPs on serum levels of human telomerase reverse transcriptase (hTERT) has not been explored before in any population. The aim of this study was to: study the influence of TERC SNPs on LTL, levels of [hTERT], indices of obesity and explore the potential associations with type 2 diabetes mellitus [T2DM].

Methods: In a study on 225 T2DM patients and 245 age and sex matched controls, we used Allelic Discrimination (AD) genotyping to determine near TERC SNPs (rs12696304 and rs16847897). Fasting [hTERT], adiponectin, Insulin, Myeloperoxidase [MPO], and [LTL] were also measured. Body Mass Index [BMI], and waist circumference (WC) were also recorded and subjects were classified on the basis of the degree of obesity. Body fat percentage [BF%] was measured using Bioimpedance analysis [BIA]. Insulin resistance [IR] was assessed using HOMA-IR calculator.

Results: [C/C] genotype of SNP rs16847897 was significantly associated with telomere shortening [OR=1.6, p=0.004] and lower levels of hTERT [OR=0.4, p=0.006]. Nevertheless, [C/C] genotype was significantly associated with higher BMI [OR=2.2, p=0.006] [OR=2.3, p=0.007] and BP’s [OR=2.0, p=0.005]. However, [C/C] genotype SNP rs16847897 was associated with hypo-adiponectemia [OR=0.6, p=0.006]. We found that [G/G] genotype of SNP rs12696304 was significantly associated with shorter telomeres [OR=1.5, p=0.004], lower levels of hTERT [OR=0.7, p=0.006] and hypo-adiponectemia [OR=0.5, p=0.008]. [G/G] genotype of SNP rs12696304 was associated with higher anthropometric measures such as BMI [OR=1.2, P=0.006], WC [OR=5.3, P=0.004] and BMI [OR=1.9, p=0.003]. Binary logistic regression showed that: [C/C] genotype of SNP rs16847897 and [G/G] genotype of SNP rs12696304 were significantly associated with higher T2DM risk [OR=1.7, p=0.004]. Carriers of haplotype [CG] had significantly higher [p<0.0001] BMI compared to the other two identified haplotypes [CC] and [GG] [BMI: 20.8±8.2 Kg/m2 vs. BMI: 26.9±4.9 Kg/m2 and BMI: 28.7±5.3 Kg/m2]. Similar trends were observed for WC and BF%. Additionally, telomere lengths were significantly the shortest and hTERT levels were the lowest in [CC], LTL: 0.8±0.1; hTERT: 21.8±5.5 ng/mL]] haplotypes compared to the other haplotypes [CC, LTL: 1.0±0.1; hTERT: 23.7±6.9 ng/mL] and (GG, LTL: 1.5±0.1; hTERT: 28.1±5.4 ng/mL)]. On the other hand, levels of MPO were significantly higher in haplotype [CG], MPO: 6.6±1.7 ng/mL compared to other two haplotypes [CC, MPO: 3.9±0.4 ng/mL] and [GG, MPO: 4.1±0.4 ng/mL]). We also found that [CG] haplotype was associated significantly with higher risk of T2DM [OR=1.5, p=0.006] and IR [OR=2.0, p=0.03].

Conclusions: We provide insights into genetic determination of a structure that is critically involved in genomic stability. Given the importance of telomeres in nuclear and cellular function and the central role of telomere length in determining telomere function; our findings could have broad relevance for both normal and pathological age associated processes.
mL; Insufficient 11-29 ng/mL and Adequate >30 ng/mL. Data were tabulated and subjected to analysis using the Statistical Package for Social Science (SPSS) version 17.0.

Results: 74.4% of indoor anesthesia personnel and 76.6% of outdoor workers had serum 25-OHD concentrations <10 ng/mL. 20.8% of anesthesia personnel and 23.4% of outdoor workers had serum 25-OHD concentration levels 10-20 ng/mL. 4.8% of personnel had serum 25-OHD concentration levels 21-30 ng/mL. There was no significant difference in the mean serum 25-OHD level between two groups (Anesthesia group: 9.8±8.89 ng/mL, Control group: 8.2±1.64 ng/mL) (p<0.05).

Conclusion: This study in Ankara suggests that significant proportions of the study populations had very low vitamin D levels at the end of winter. Vitamin D deficiency/insufficiency is common among indoor and outdoor workers. Anesthesia personnel do not have a significant higher Vitamin D deficiency/insufficiency risk. As we have seen, UV irradiance is not the only determinant of vitamin D status. Individuals living at lower latitudes in relatively sunny environments are also at risk of vitamin D insufficiency. Vitamin D supplementation may be suggested in all groups in Ankara, including those with the highest sun exposure.

**Increased Cortisol and NADPH Production in Magnesium Deficient Hepatocytes: Implicated in the Onset of Insulin Resistance and Obesity,**

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Most of the clinically quantifiable liver functions take place within the hepatocytes (80% of liver cells). The current western diet is approximately 35% deficient in magnesium (Mg2+). Subnormal Mg2+ concentrations have been reported in both diabetes and obesity, but no clear-cut cause-effect mechanism has been stated to elucidate the onset of these pathological conditions in Mg2+ deficiency. At the cellular level, Mg2+ is highly concentrated within organelles including the endoplasmic reticulum (ER), in which 15-20 mM [Mg2+]ER has been measured. Hexose 6-phosphate dehydrogenase (H6PD), the reticular counterpart of the cytosolic G6PD, is the main NADPH generating enzyme within the ER of the hepatocyte and is regarded as an ancillary enzyme in pre-receptor glucocorticoid activation. In the present study, we report that by modulating glucose 6-phosphate entry into the ER of HepG2 cells, Mg2+ also regulates the oxidation of this substrate via H6PD. This regulatory effect is dynamic as glucose 6-phosphate entry and oxidation can be rapidly down-regulated by increase in NADPH production via H6PD in Mg2+ deficient conditions.

**Comparison of IFA and RIA based assays for measuring adrenal autoantibody response**

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Background: The diagnosis of autoimmune Addison’s disease (ADD), a primary adrenal insufficiency, depends on demonstrating inappropriately low cortisol production and high titters of adrenal cortex autoantibodies (ACAAs) or 21-hydroxylase (21-OH) autoantibodies. ACAAs are determined using an immunofluorescence assay (IFA), while 21-OH autoantibodies are detected with a radioimmunoassay (RIA). In IFAs, response against 21-hydroxylase (21-OH) accounts for majority of the immunoreactivity, but antibodies against two other steroidogenic enzymes (17α hydroxylase [17-OH] and SCC [P450scc]) also contribute. A sensitive and convenient RIA is available to measure anti 21-OH antibody using recombinant 125I-labelled 21-OH expressed in yeast. Discrepancy between ACA IFA and 21-OH RIA test results was reported among individuals with endocrine autoimmune diseases often associated with adrenal insufficiency. We evaluated concordance between ACA IFA and 21-OH RIA results in a large set of samples received for routine adrenal antibody testing. Methods: De-identified residual specimens (n=280) originally submitted to Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) for routine adrenal antibody testing by either IFA (n=140) or RIA (n=140) were re-tested with both assays. Also included in the analysis were 1) results for an additional 264 specimens submitted for both testing with assays (ACA IFA and 21-OH Ab RIA), and 2) sera positive for mitochondrial antibodies. The ACA IFA was performed using monkey adrenal tissue (MarDx Diagnostics, Inc., Carlsbad, CA) and FITC-labeled goat antibodies to human IgG (Inova Diagnostics, Inc., San Diego, CA). Anti 21-OH testing was performed with a commercial RIA (Kromus Inc., Star, ID) employing 125I-labeled 21-OH produced in yeast. Anti-mitochondrial antibodies (AMAs) were detected by IFA using rat kidney tissue (MarDx Diagnostics) and by Quantitec™ ELISA (Inova Diagnostics) employing recombinant antigen (MIT3). Results: The two assays yielded concordant results in 460 (83%) of the 554 samples, including 328 with negative and 132 with positive results. 94 samples were discordant. Samples with low positive results were the main contributors for IFA/RIA discordance: among 55 RIA+/IFA- samples, 49 showed RIA values close to the cut off and only 6 samples had values >10 U/mL. This discrepancy could be due to the fact that some of 21-OH epitopes recognized in RIA were “hidden” or not present in IFA substrate. Among 29 RIA-/IFA+ samples, most (18/29) had titers of 1:10 and only 4 had titers >1:40. The presence of other antibodies, either to steroid-producing cell antibodies or anti-mitochondrial antibodies, could cause these discrepancies. Presence of mitochondrial antibodies was identified in one sample. Conclusion: Low-positive samples are the major contributors to discrepancies between IFA and RIA results on adrenal antibody testing. The presence of anti-mitochondrial antibody may interfere with adrenal antibody testing by IFA.

**Extreme Physical Stress Stimulates Bone Marrow-derived Circulating Stem/Progenitor Cells that Mediate Tissue Repair: Possible Clinical Implications**

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Background: Autologous progenitor cells represent a promising option for regenerative cell-based therapies. Endothelial progenitor cells (EPCs) participate in vascular repair and angiogenesis, while circulating bone marrow-originated fibrocytes represent multipotent cells mediating tissue repair and remodeling after injury. Aging and cardiovascular risk factors, such as diabetes, however, affect circulating endothelial and bone marrow-derived progenitor cells, limiting their therapeutic potential. The “Spartathlon” ultradistance foot race (246Km continuous, prolonged, brisk exercise for up to 36h), is associated with profound physical strain, which renders it an ideal model of prolonged severe physical stress. The runners endure dramatic systemic and inflammatory changes, as their immune system functions intensively to cope with heart and skeletal muscle and other organ damage secondary to excessive physical strain. We hypothesized that this type of exercise might stimulate release of EPCs and other bone marrow-derived cells.
Athletes and Methods: We investigated the effect of physical stress on the number of circulating EPCs and fibrocytes, along with circulating molecules indicative of endothelial dysfunction and adipose tissue-derived proteins, in 20 “Spartathlon” athletes before, at the end and at 48 h post-race. The EPCs were obtained by culturing peripheral blood mononuclear cells (PBMC) under endothelial cell conditions (EndoCult) and were measured as colony-forming units (CFUs). Circulating fibrocytes were cultured from PBMCs in IMDM medium supplemented with IL-5 and M-CSF and identified as CD45+/CD14+/CD34low/ColllA1+ fibroblastic cells. We also determined the plasma levels of endothelial dysfunction molecules E- and P-selectins, soluble Intercellular Adhesion Molecule-1 (sICAM-1), soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1), and thrombomodulin (TM), along with adipose tissue-derived proteins leptin, adiponectin (ADPN), lipocalin-2 (NGAL), Retinol Binding Protein-4 (RBP-4), Plasminogen Activator Inhibitor (PAI-1), Macrophage Migration Inhibitory Factor (MIF), IL-8 and Macrophage Chemottractant Protein 1 (MCP-1) by means of immunoenzymatic techniques. Results: Circulating EPCs increased by nearly ten-fold in peripheral blood at the end of the “Spartathlon” race (from 48±15 cells/ml to 464±36 cells/ml) and they remained increased (420±28 cells/ml) even at 48h post-race (p<0.5). Plasma levels of endothelial dysfunction molecules showed different patterns of responses: E-selectin, sICAM, sVCAM and thrombomodulin were increased significantly at the end of the race and returned to pre-race levels 48 h post-race, (p<0.06). Similarly, the adipose tissue-derived proteins NGAL, IL-8 and MCP-1 showed significant increases at the end of the race and returned to pre-race levels 48 h post-race, (p<0.5). Conclusions: Our study demonstrates that acute inflammatory tissue damage induced by exhaustive exercise increases EPCs but not fibrocytes. Given the ability of EPCs to promote angiogenesis and vascular regeneration and the association of fibrocytes with tissue fibrosis after persistent inflammation, we conclude that this kind of cell mobilization may serve as a physiologic repair mechanism in acute inflammatory tissue injury and a source of potential cell therapies in the near future. Furthermore, this study shows different patterns of adipose tissue-derived protein response to the systemic effort and inflammatory changes.

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CDC Standardization Programs- Testosterone, Estradiol, and Vitamin D

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Laboratory measurements are critical in patient care and public health decision making. However, the accuracy and reliability of these measurements present appropriate detection, treatment and prevention of diseases. The aim of CDC Standardization Programs is to standardize clinical measurements which ensure that accurate and comparable measurements are obtained regardless of the measurement procedure, location, and time. To achieve this goal, the CDC Standardization Programs are providing a comprehensive range of services and programs such as Reference Services, Standardization-Certification Programs, and Accuracy-based Quality Assurance. Monitoring Services for testosterone (T), estradiol (E2), and vitamin D [25(OH)D]. As part of the Reference Laboratory Services the CDC has established higher order reference measurement procedures for T, E2, 25(OH)D2 and 25(OH)D3 in serum using LC-MS/MS. These measurement procedures are traceable to primary reference materials and to JCTLM certified reference measurement procedures. Using these reference methods, CDC assigns target values to sera used in its certification programs and by outside partners such as clinical and research laboratories, assay manufacturers, and proficiency testing providers. These materials are used for method comparisons, calibration, and trueness controls. CDC Standardization CDC Standardization-Certification Programs are operating for T and E2 with the Hormone Standardization (HoSt) Program and total 25(OH)D with the Vitamin D Standardization-Certification Program (VDSCP). In both of these programs, quarterly blinded challenges are performed. Bias and imprecision assessments using established protocols and final assessment are made using criteria derived from biological variability. At present, 17 participants are enrolled in the HoSt-T Program (established in 2010) and 23 in VDSCP (established in 2013). Participants include clinical, academic, and pharmaceutical laboratories as well as manufacturers. Approximately 85% of participants have met the established criteria. Successful laboratories are published on the CDC website (http://www.cdc.gov/labstandards/cs.html). Over the past 4 years the CDC has provided 97 calibration verification serum sets to requesters and has had 85 enrollments in the HoSt-T Program, which include many reenrollments. While participation has increased the success rate of participants has continued to improve as well. The testosterone HoSt Program has increased success rates by participants over the past 3 years from 79% in cycle 1 to 100% in cycle 3. CDC Hormone Standardization Programs are endorsed and supported by key stakeholders such as the Partnership for Accuracy in Hormone Testing (PATH) and its affiliated organizations (i.e., AACC, The Endocrine Society, and American Urology Association). Furthermore, it collaborates with these organizations to further improve testing for other hormones such as thyroid hormones.

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Quantifying Insulin-like Growth Factor-1: Inter-assay Variation Remains an Issue

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Background: As the main mediator of the somatotropic effects of growth hormone (GH), an accurate measurement of human insulin-like growth factor-1 (IGF-1) is required for the diagnosis and management of GH secretion disorders. However, the standardized measurement of IGF-1 continues to suffer from inter-assay variability, which may lead to inaccurate patient case decision making. In early 2013, the receipt of a vendor notification stating that the IGF-1 reagent lots deployed in our laboratory positively shifted patient median values prompted us to validate and ultimately deploy an alternate vendor’s IGF-1 platform for patient testing. Recently, the primary vendor resumed IGF-1 reagent supply, triggering a secondary validation of these reformulated lots. These two studies specifically examined the inter-assay variability of IGF-1 measurements and the relative analytical performance of each test.

Methods: Linearity, intra- and inter-day precision, accuracy and sample carry-over were validated for IGF-1 measurements using the IDS-iSYS (Immunodiagnostic Systems) and the reformulated Immulite 2000 (Siemens Healthcare Diagnostics) assays, respectively. Patient correlation studies between the IDS-iSYS and the original and reformulated Immulite 2000 reagents were also respectively performed.

Results: The IDS-iSYS and reformulated Immulite 2000 assays had linear ranges of 10 to 1200 ng/mL (R² = 0.998, slope = 0.973) and 20 to 1700 ng/mL (R² = 0.999, slope = 1.02), respectively. At IGF-1 concentrations of 30.8, 249.1, 930.3 ng/mL and 45.0, 67.5, and 227.0 ng/mL the intra- and inter-day precision (%CV, N=20) of the IDS-iSYS and reformulated Immulite 2000 assays did not exceed 4.6% and 7.5%, respectively. The relative error (%RE) of the IDS-iSYS and Immulite 2000 methods respectively ranged from -8.4% to 1.5% and -3.5% to 7.5% for these precision studies. No significant carry-over was observed on either platform. Patient sample comparisons between the IDS-iSYS and the original Immulite 2000 formation showed significant bias (Deming regression: y = 0.739+35.87, N=94, R²=0.988). This method bias was exacerbated at IDS-iSYS derived IGF-1 concentrations >300 ng/mL (Deming regression: y = 0.616×114.88, N=15, R²=0.981, IDS-iSYS range=256.3 to 770.1 ng/mL, Immulite 2000 range = 307.0 to 1128.0 ng/mL), relative to lower IGF-1 concentrations (Deming regression: y = 0.854×14.73, N=79, R²=0.976, IDS-iSYS range= 42.8 to 290.7 ng/mL, Immulite 2000 range = 31.5 to 351.0 ng/mL). Interestingly, this bias was less significant when patient results obtained with the reformulated Immulite 2000 reagents were correlated to those obtained with the IDS-iSYS (Deming regression: y = 1.082×0.9, N=60, R²=0.989, Immulite 2000 range = 25.0 to 352.0 ng/mL, IDS-iSYS range=31.0 to 352.0 ng/mL), although fewer samples with IGF-1 concentrations >300 ng/mL were included in this cohort.

Conclusion: Although the reformulated Immulite 2000 and IDS-iSYS IGF-1 assays offer acceptable analytical and clinical performance, a significant bias was noted with the original Immulite 2000 formulation. This difference was observed despite both Immulite formulations being traceable to the reference standard (NIBSC 1° IRR 87/518). The IDS-iSYS assay is traceable to NIBSC 02/254. Laboratories should be aware that inter-assay IGF-1 variability must be carefully examined and its impact on the diagnosis and management of GH deficiency and acromegaly considered when testing platforms are changed.

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Development of a Biochip Based Immunooassay for Quantification of Total Beta hCG in Serum

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Background: Human chorionic gonadotropin (hCG) is a member of the glycoprotein hormone family. It is heterodimeric and the alpha-subunit of hCG (92 amino acids) is identical to that of LH, FSH and TSH. The beta-subunit of hCG in Serum

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for the biological activity of this hormone. hCG interacts with the LH/hCG receptor and stimulates and maintains the corpus luteum after fertilization so it will not degenerate. The corpus luteum of pregnancy produces increasingly greater amounts of estrogen and progesterone for an additional ten weeks until the placenta takes over the secretion of these steroid hormones. This study reports the development of a biochip based immunoassay for the determination of total beta hCG in serum. Methods: A sandwich chemiluminescent biochip based immunoassay applied to the Evidence Investigator analyser was employed. The capture antibody was immobilised and stabilized on the surface of the biochip and detector antibody was conjugated to HRP. Chemiluminescent signal was detected by digital imaging technology. The intensity of the signal is proportional to the analyte concentration in the sample. A correlation study was conducted using a commercially available in-house immunoassay. Results: The assay was target specific showing <1% cross-reactivity with FSH, LH and prolactin and <1% recovery of hCG. The limit of detection was 0.914 mIU/mL for an assay range 0-2500 mIU/mL and the limit of blank was 0.388 mIU/mL. In the correlation study 80 serum samples were tested and the following linear regression equation was achieved versus another available immunoassay: y=1.513x-185.07; r=0.984. Conclusion: This evaluation indicates applicability of the developed biochip based immunoassay for the detection of total beta hCG in serum. This represents a new analytical tool for the detection of pregnancy in test settings.

Performance Characteristics of Six Automated 25-Hydroxyvitamin D Assays: Mind Your 3’s and 2’s
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Objective: Analyze the performance of 6 automated total 25-hydroxyvitamin D assays using 25(OH)D3/D3 and 25(OH)D3 only samples. Methods: Access 2 and DxI 800 (Beckman Coulter*), ARCHITECT 2000i (Abbott Diagnostics), ADVIA Centaur XP (Siemens), Liaison XL (DiaSorin) and Modular E170 (Roche Diagnostics) assays were evaluated for imprecision, method comparison and concordance. Imprecision used commercial control material tested in duplicate twice daily for 5 days. Method comparisons used residual serum samples with endemic D2 and D3 (n=50) or D3 only (n=86). Comparisons with all 136 samples were intended to simulate real-world laboratory testing. Results: were compared to an in-house LC-MS/MS method (traceable to NIST SRM 972) using Passing-Bablok regression and Bland-Altman bias plots. Acceptability criteria were coefficient of variation (CV) ≤10% and bias ≤15%. Results: Imprecision was acceptable for all assays except E170 and Centaur (both CV 11%). Regression analysis of all samples in comparison to LC-MS/MS demonstrated under-recovery for ARCHITECT, DxI, E170 and Liaison assays (slopes 0.868, 0.983, 0.912, 0.834) while Access and Centaur over-recovered (slopes 1.013, 1.030). All correlation coefficients were below 0.95. Compared to D2/D3 samples, E170 and Centaur showed the greatest improvement in slope without D2 while Liaison was unaffected. Also, E170 under-recovered with D2/D3 and over-recovered in the absence of D2. Access, Centaur and Dxl assays exhibited the opposite effect. Constant bias for all samples ranged from -3.3 (Centaur) to 1.7 ng/mL (ARCHITECT). Intercepts improved without D2 present for all assays except ARCHITECT and E170. Centaur constant bias improved the most in the absence of D2. Testing all samples, Centaur had the lowest overall bias (2%) and E170 (20%) and Liaison (22%) exceeded acceptable criteria. Testing D2/D3 samples, Dxl and Access had the lowest bias (4%); ARCHITECT (26%), E170 (36%) and Liaison (29%) exceeded acceptable criteria with these samples. In the absence of D2 the Liaison still exceeded this limit (18%), the ARCHITECT had the lowest bias (1%) and E170, Centaur and Access were comparable to each other (8-9%). All assays over-recovered when analyzing vitamin D deficient samples (<20 ng/mL, n=31), with E170 (20%) and Liaison (19%) exceeding bias criteria. Concordance with LC-MS/MS at 20 ng/mL ranged from 77% (Centaur) to 89% (DxI). ARCHITECT, E170 and Liaison concordance improved without D2. Overall, Access and Dxl had slopes close to 1 and acceptable bias for all sample groups. Liaison had the lowest slopes and was not affected by D2. While ARCHITECT slope and intercept were not greatly affected by D2, bias and concordance improved without D2 present. E170 and Centaur assays were most affected by D2, based on improvements in slope, intercept or bias when D2 was absent. Conclusions: It is important to consider the effects of D2 and D3 on individual assay performance. Assessing performance using total vitamin D may mask possible interferences in supplemented populations.

Quantification of Anti-Müllerian Hormone by the AnshLabs picoAMH ELISA Assay
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Background: Anti-Müllerian hormone (AMH) is responsible for regression of the female ductal system during embryonic development. It is produced by the male testes until puberty and the female granulosa cells until menopause. A highly sensitive AMH assay is ideal for investigation of infertility, menopause, ovarian reserve or monitoring granulosa cell tumors post therapy. The picoAMH ELISA kit (Ansh Labs, Webster, TX, USA) is a new quantitative immunoassay that detects ultra-low concentrations of AMH in human serum. Here, we describe the analytical performance of the picoAMH assay.

Methods: Accuracy studies were performed using manufacturer’s controls (65 and 185 pg/mL) and patient pools (201 and 404 pg/mL) assayed in duplicate or triplicate once daily for 10 days. Dilution imprecision was tested using 1:10 and 1:100 dilutions of serum pools assayed in triplicate once daily for 5 days. Limit of blank (LOB) and limit of detection (LOD) were assessed using the blank and 6.3 pg/mL calibrators. Linearity was determined by serially diluting a high AMH sample in blank calibrator to create 5 samples tested in duplicate. Recovery was evaluated by adding the highest 2 calibrators to patient samples (84 and 202 pg/mL). Temperature stability was determined by storing 2 specimens (99 and 301 pg/mL) ambient for 24 hr, 4°C for 7 days and -20°C for 3 weeks. Effects of up to 3 freeze/thaw cycles were studied. Method comparison yielded y = 0.999 x -0.226, R² = 0.99. Eleven gender-specific reference intervals were established using fresh or repository serum specimens (6 mos–7 yrs, n=2,733).

Results: Accuracy and dilution imprecision studies showed total CVs ≤6.3 and ≤8.7%, respectively. LOB and LOD were 0.81 and 3.11 pg/mL, respectively. The assay was linear to 696 pg/mL. Recovery ranged 76-101%. AMH differed <18% at all storage temperatures and <5% after 3 freeze/thaw cycles. Deming regression of the method comparison yielded y = 0.999 x -0.226, R² = 0.99. Eleven gender-specific reference intervals were established using non-parametric and robust statistics.

Conclusions: The AnshLabs picoAMH ELISA demonstrated performance close to the manufacturer claims and excellent correlation with the comparator method. This method lowered the LOD from the current 80 pg/mL to 3 pg/mL. We report gender-specific reference intervals for this assay that will be useful for clinical practice.

Signaling and steroid metabolism in human placenta from gestational diabetic subjects
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Background: Insulin and leptin receptors are known to share signaling pathways, such as JAK2/STAT-3 (Jamus kinase 2/signal transduction and activator of transcription 3), MAPK (Mitogen activated protein kinase) and PI3K (phosphoinositide 3-kinase). Both positive and negative cross-talk have been previously found in different cellular systems. Gestational diabetes (GDM) is a pathophysiological state with high circulating levels of both insulin and leptin. We have previously found that these three signaling pathways are activated in placenta from GDM patients to promote translation, involving the activation of leptin receptor. Now, we tested the hypothesis that both insulin and insulin receptors might contribute to this activation in a positive way that may become negative when the system is overactivated.

Methods: To answer this question we studied the activation of insulin and leptin receptors in placenta from gestational diabetic subjects using specific antibodies activated in placenta from GDM patients to promote translation, involving the activation of leptin receptor. Now, we tested the hypothesis that both insulin and insulin receptors might contribute to this activation in a positive way that may become negative when the system is overactivated.

Conclusions: The AnshLabs picoAMH ELISA demonstrated performance close to the manufacturer claims and excellent correlation with the comparator method. This method lowered the LOD from the current 80 pg/mL to 3 pg/mL. We report gender-specific reference intervals for this assay that will be useful for clinical practice.
stimulation with leptin showed negative effect. Similarly, trophoblastic explants from GDM placenta, which presented high signaling levels, had a negative signaling effect when further incubated in vitro with leptin.

Conclusions: Insulin and leptin receptors have positive effects on signaling, contributing to high signaling levels in placenta from GDM, but insulin and leptin have negative effects upon overstimulation.

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Ultrasonic Luteinizing Hormone Assay on the MesaScale Discovery Platform


Background: Luteinizing Hormone (LH) or Lutropin is a glycoprotein composed of α and β subunits secreted by the anterior pituitary gland after stimulation by gonadotropin-releasing hormone (GnRH). In children, LH is measured as an aid in the diagnosis of gonadal disorders such as central precocious puberty (CPP) and delayed puberty. Current automated immunoassays are sensitive to about 0.1 IU/L. The assay is a sequential two-site electrochemiluminescence laboratory developed test. A monoclonal biotinylated LH capture antibody is added to a streptavidin coated plate, incubated for 30 min and washed to remove unbound antibody. Sample is added and incubated overnight at 4°C. After washing, a SULFO-TAG™ labeled detection antibody is added. After 2 h the plate is washed and counted on the MSD platform.

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Preanalytical validation of a serum normetanephrine, metanephrine and 3-methoxytyramine assay

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Background: Analysis of metanephrine (NM), normetanephrine (NMM) and 3-methoxytyramine (3MT) in plasma or serum has recently replaced the assay in many laboratories for the diagnosis of pheochromocytoma. The aim of this study was to validate preanalytical factors of serum MN, NMN and 3MT assays.

Methods: We used five laboratories from apparently healthy adult volunteers to study sample stability (n=25), sampling device (n=13), postprandial effect (n=7), intra-individual within-day variation and interday variation (n=7). Samples (200 μL) with [1H3]-labeled internal standards were extracted with Oasis® WCX µElution plate (Waters), washed with water, methanol and 0.1% formic acid in acetonitrile and eluted with 2 μL of 2% formic acid in 95% acetonitrile-5% water. The eluent (25 μL) was analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) employing an Agilent 1200 liquid chromatograph (Agilent Technologies), a 4000 QTRAP mass spectrometer (AB Sciex), and an Atlantis HILIC Silica 50x2.10 mm column (Waters). The LOQ of the assay was 0.025 nmol/L, the intra-assay CV was <7.2%, the inter-assay CV was <8.3%, and the linear range 0.025-5 nmol/L for MN, NMN and 3MT. Paired t-test was performed by Analyse-it for Microsoft® Excel 2003. Results: Serum MN and MN were stable (concentration changed <20%) for at least 7 days at room temperature and at 4°C, for 12 weeks at -20°C. MN was stable during 1 and MN at least during 4 freeze-thaw cycles. No valid stability data of serum 3MT could be obtained because the concentrations were below the detection limit in the majority of our samples. MN and 3MT concentrations were lower (p<0.032) in samples drawn into Li-heparin plasma tubes (mean 0.41 and 0.03 nmol/mL, respectively), Venosafe 60 USP U Lithium Heparin tube, Terumo) than in samples drawn into glass tubes (0.49 and 0.05 nmol/mL, respectively), clotting catheter tubes (0.47 and 0.04 nmol/mL, respectively) and SST™ II Advance gel tubes (0.47 and 0.04 nmol/mL, respectively). All serum tubes were from Vacutainer. On contrary, MN was the highest in Li-heparin plasma (0.18 nmol/L), but the difference was significant only as compared to serum drawn into catheter tubes (0.17 nmol/L, p<0.0165). A regular breakfast meal had no effect on serum MN, NM or 3MT concentrations (p<0.075 for all). There was no difference (p=0.066) in MN and 3MT concentrations in samples drawn at 8 a.m. (0.48 and 0.03 nmol/mL, respectively), noon (0.51 and 0.04 nmol/mL, respectively) and 4 p.m. (0.45 and 0.04 nmol/mL, respectively). However, MN concentration was 0.16 nmol/mL at 8 a.m., 0.17 nmol/mL at noon and 0.19 nmol/mL at 4 p.m. (p=0.0304). The mean intra-individual within-day variation of MN, NM and 3MT was 13% (range 7%-23%), 13% (range 3%-13%) and 22% (range 9%-36%), respectively.

Conclusions: To minimize assay variation due to preanalytical factors, we suggest that samples be transported to the laboratory at room temperature but stored frozen. Only 1 freeze-thaw cycle should be allowed before analysis, serum instead of Li-heparin plasma should be used, sampling should occur before noon and no fasting before sampling is required.
**Development of a sensitive Dried Blood Spot Anti-Mullerian Hormone (AMH) ELISA**

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**Background:** The aim of this study was to develop a highly sensitive and simple dried blood spot human AMH ELISA to assess ovarian reserve.

**Relevance:** AMH has been reported to be strongly associated with age, antral follicle counts (AFC), FSH and has emerged as a clinically useful biomarker of ovarian reserve. Recently, there have been concerns related to AMH stability in serum/plasma and complement interferences affecting the end result. This has generated numerous debates and publications related to reproducibility of AMH measurements and impact of pre-analytical sample handling. Dried blood spot specimens stability makes it a practicable alternative to venous blood. It opens new possibilities in AMH testing, such as comparison of historical to current patient results; simplified blood sampling for patients in remote locations or for those who are homebound.

**Methods:** A three-step, sandwich-type enzymatic microplate assay has been developed to measure AMH levels in two 7.9 mm dried blood spot disc in less than 6 hours. The assay measures human AMH and uses stabilized recombinant human AMH as calibrators (7-1000 pg/mL). This method uses a drop of whole blood collected on filter paper from a simple finger stick. The sample is eluted from the dried blood spot in an extraction solution and is added directly to the well. The assay measures the bio-essential AMH and does not exhibit interference by hemocytin in the extracted spot.

**Results:** Ansh Labs DBS AMH ELISA (AL-129), when compared to Ansh Labs US AMH ELISA (AL-105) using 56 matched serum and dried blood spot samples in the range of 62-18443 pg/mL yielded a correlation coefficient of 0.98 (p < 0.0001) and a slope of 0.96 with an intercept of -7.56 pg/mL. DBS AMH ELISA (AL-129) when compared to Ansh Labs picoAMH ELISA (AL-124) using 65 matched serum and dried blood spot samples in the range of 5-5240 pg/mL yielded a correlation coefficient of 0.99 (p < 0.0001) and a slope of 1.02 with an intercept of -4.7 pg/mL. Serial dilution of seven extracted dried blood specimens (5000-11000 pg/mL) in the sample diluent showed an average recovery of 87-105%. Total imprecision, calculated on 3 controls over 40 runs, 2 replicates per run, was 5.84% at 22.58 pg/mL, 3.15% at 86.51 pg/mL and 4.34% at 373.18 pg/mL. The functional sensitivity of the assay calculated at 20% CV was 3.9 pg/mL.

**Conclusion:** A highly simplified, sensitive, specific and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age. The DBS results are comparable to serum based assays. The specimen stability, ease and low cost of collection and transportation makes it a very attractive sample type for epidemiologic and other research studies.

**A-230**

**Nonalcoholic Steato-hepatitis (NASH) in Type 2 Diabetes: Serum Body Fat-normalized Plasma Leptin Level is a Predictor of Serum Alanine Aminotransferase (ALT)**

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**Background:** Leptin is a multifunctional hormone which may be involved in the pathogenesis of type 2 diabetes mellitus (T2DM) and its complications. Anti-steatotic function of leptin is well demonstrated in animal studies. Nonalcoholic steato-hepatitis (NASH) pathogenesis of type 2 diabetes mellitus (T2DM) and its complications. Anti-steatotic effects of leptin and leptin resistance in type 2 diabetes mellitus (T2DM) has raised the interest in the relationship between leptin and liver diseases. The aim of this study was to investigate this potential effect using an established mass balance model for AG and varying the AG reabsorption fraction to the same degree of the effect on glucose reabsorption caused by the new reabsorption inhibitors.

**Methods:** We used a two-compartment AG mass balance model previously described. The model predicts an exponential decrease in plasma AG when fe is increased, sensitive, speciﬁc and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age.

The model predictions are an exponential decrease in plasma AG when fe is increased, sensitive, specific and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age.

**A-231**

**Predicted decrease of plasma 1,5-anhydroglucitol (AG) in presence of inhibitors of glucose reabsorption (SGLT2 inhibitors): potential utility of AG as a primary marker of drug effect**


**Background:** Renal reabsorption of glucose under conditions of normoglycemia is essentially 100%. Drugs that inhibit renal glucose reabsorption, via inhibition of the main renal glucose transporter (sodium-glucose transporter-2, or SGLT2), have recently been approved for use in the U.S. for treatment of Type 2 diabetes (e.g., Invokana). Renal reabsorption of plasma 1,5-anhydroglucitol (AG, 1-deoxyglucose), an unregulated, non-metabolizable glucose analogue derived from diet, is normally >99%; normal plasma AG represents a balance between slow rates of input (5 mg/d) and excretion. In diabetes, plasma AG is often substantially decreased due to accelerated renal loss that occurs when glucose concentration is high enough to saturate reabsorption capacity (viz., under conditions of glucosuria). Correspondingly, plasma AG is likely to be directly affected by drugs that inhibit glucose reabsorption. Our objective was to examine this potential effect using an established mass balance model for AG and varying the AG reabsorption fraction to the same degree of the effect on plasma AG for following a step increase in fe from 0.01 to 0.95.

**Results:** The model predictions are an exponential decrease in plasma AG when fe is increased, sensitive, specific and reproducible dried blood spot AMH assay has been developed to assess ovarian reserve in females of reproductive age.

**Conclusions:** SGLT2 inhibitors are predicted to produce a rapid and substantial decrease in plasma AG. The effects of SGLT2 inhibitors would obviate the usual accelerated renal loss that occurs when glucose concentration is high enough to saturate reabsorption capacity (viz., under conditions of glucosuria). Correspondingly, plasma AG is likely to be directly affected by drugs that inhibit glucose reabsorption. Our objective was to examine this potential effect using an established mass balance model for AG and varying the AG reabsorption fraction to the same degree of the effect on plasma AG caused by the new reabsorption inhibitors.
Objective: The goal of this study was to evaluate the impact of systemic RAS inhibition (AngII receptor blocker, ARB), and the attendant suppression of renal oxidative stress, on the renal tissue RAS during the normoalbuminuric stage of DM.

Methods: Four groups of rats (n=5 per group) were examined: 1) STZ group: rats studied 2 wks after induction of DM by streptozotocin injection (STZ, 65 mg/kg i.p.), 2) Sham group: rats receiving the STZ vehicle, 3) STZ+TLM group: STZ rats treated with telmisartan (TLM, an ARB; 10 mg/kg/day) in chow for 2 wks, and 4) Sham+TLM group: TLM-treated Sham rats. In each rat, blood glucose, blood pressure and glomerular filtration rate (GFR) were measured. We quantified the following parameters in renal cortex: 3-nitrotyrosine (3-NT) production (an oxidative stress marker; by HPLC), AngII levels (by RIA), (P)RR expression, and expression of both angiotensin type-I and type-2 receptors (AT1R and AT2R by western blotting).

Results: Similar to previous reports, blood glucose levels were higher in STZ and STZ+TLM groups than in Sham and Sham+TLM groups. Blood pressure did not differ among groups. GFR was increased in STZ group compared with Sham (P<0.05), but however, TLM suppressed this phenomenon (P<0.05; STZ+TLM). Renal cortical 3-NT production was increased in STZ compared with Sham (P<0.05); however, TLM did not alter either parameter in the Sham group. Renal cortex AT1R expression was increased in the STZ group (155±6% of Sham; P<0.05), and further increased by TLM-treatment (182±10% of Sham; P<0.05).

Conclusions: During the normoalbuminuric stage of DM in the rat, the renal cortex exhibits upregulation of major components of the intrarenal RAS (AT1R, AT2R and (P)RR) without a change in tissue AngII levels. The DM-induced changes in AT1R and (P)RR expression are prevented by systemic ARB blockade, and may arise via oxidative stress. These observations indicate that the renoprotective effects of ARB may involve not only an antioxidant effect but also effects that rely on suppression of the intrarenal RAS.

Comparison of Immunoassays to Mass Spectrometry for Free and Total Testosterone in Men, Women, and Children

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Background: Circulating testosterone may be bound to albumin, sex hormone binding globulin (SHBG) or remain free. Measurement of these various forms of testosterone provides an overall assessment of androgen status and aids diagnosis of several conditions in men, women, and children. Study objectives were to compare 5 commercially available immunoassays to mass spectrometry for free and total testosterone in adults and children.

Methods: Residual serum samples from men (n=150), women (n=100), boys (n=25), and girls (n=25) were obtained after completion of clinical testing for total testosterone (TT) by liquid chromatography tandem mass spectrometry (LC-MS/MS). Free testosterone (FT) was determined in men using equilibrium dialysis (ED)/LC-MS/MS. All samples were further tested for TT and SHBG by the Abbott ARCHITECT c8200, Siemens Advia Centaur and IMMULITE 2000, Beckman Coulter DxL, and Roche Modular E170. Albumin was measured using the Abbott ARCHITECT c8200 and Roche c702. FT was calculated using the Vermeulen equation. For women, boys, and girls, calculated FT by immunoassays was compared to calculated FT using TT by LC-MS/MS.

Results: Comparisons using Deming regression for TT and FT in men and women are provided (Table). For boys and girls, slopes for TT ranged from 0.72 (IMMULITE) to 1.04 (IMMULITE) and 0.72 (ARCHITECT) to 1.04 (ARCHITECT) and 1.04 (ARCHITECT) to 1.04 (ARCHITECT), respectively. Overall, more immunoassays under-recovered in men and women and over-recovered in boys and girls for both TT and FT. The average of absolute percent bias was highest in boys for both TT (92.4%) and FT (91.8%) compared to men (8.7% and 10.8%, respectively).

Conclusions: Consistent biases were not observed amongst methods and populations evaluated. Challenges with accurately measuring testosterone appear to remain in some immunoassays, but not all.
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