# Tuesday, July 31, 2018

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

Endocrinology/Hormones

## A-166

### Testosterone levels evaluation in a cohort of 1 million Brazilian women.

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**Background:** Testosterone is the most important male hormone and it is present in women in low concentrations. Currently low dose supplementation of this hormone in women has been designated due to beneficial effects. However, the indiscriminate use and without medical supervision can cause risk of toxicity influenced by the route of administration, dose and individual sensitivity that lead to adverse effects. Although the use of androgens is controlled by prescription, access to these medicines has been increasing with unregistered formulas, herbal medicines, dietary supplements and illegal market. **Objective:** To evidence the alteration of testosterone level in clinical samples from women of different age groups during the period from 2013 to 2017. **Methods:** This is a study carried out through consultation of data collected of total testosterone test (Beckman Coulter) in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) database during the period of 2013 to 2017. The lower limit of reference range for males (175 ng/dL) was used to designate the results as altered. The women were stratified in groups by age (≤ 17 years; 18 to 45 years and ≥ 46 years). **Results:** A total of 942,625 women were evaluated from 2013 to 2017. The results showed that there was an increase in absolute number of altered results in age group of 18-45 years and a decrease in age group ≤ 17 and ≥ 46 years. **Conclusions:** Despite the lack of objective clinical data, and assuming stability in the prevalence/incidence of the main diseases associated with the elevation of testosterone in women, therefore, these results allow to infer that the possible supplementation of testosterone could be the responsible for this profile. These results help to better understand the profile of testosterone results released by the laboratory, as well as signaling to evaluate the current practices of hormonal supplementation in women.

**Table 1: Absolute number of altered results in women.**

| YEARS | TOTAL NUMBER | ALTERED RESULTS | ≤17 YEARS | 18-45 YEARS | ≥46 |
|---|---|---|---|---|---|
| 2013 | 99,593 | 2,180 (2.18%) | 167 (7.7%) | 853 (39.1%) | 1,160 (53.2%) |
| 2014 | 135,656 | 2,615 (1.92%) | 171 (6.6%) | 1,083 (41.4%) | 1,360 (52.0%) |
| 2015 | 163,531 | 3,575 (2.18%) | 239 (6.7%) | 1,596 (44.6%) | 1,740 (48.7%) |
| 2016 | 247,317 | 5,362 (2.16%) | 300(5.6%) | 2,764 (51.5%) | 2,298 (42.9%) |
| 2017 | 323,528 | 7,534 (2.32%) | 357(4.7%) | 4135 (54.9%) | 3,042 (40.4%) |

## A-167

### Evaluation of estradiol levels in patients using selective estrogen receptor modulators

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**Background**: Selective estrogen receptor modulators (SERMs) are molecules that bind agonist or estrogen receptor antagonists (ERs) in specific tissues, which allow them different clinical performances. The action as an estrogen antagonist is used in the treatment of breast cancer. Recently, this type of employment has been approved in chemoprevention in women with high risk of developing breast cancer, because it acts in a competitive way to ERs, blocking the action of estrogen, which in its turn stimulates cell division in the breast. The purpose of this study is to evaluate the profile of estradiol levels in patients using SERM. **Methods**: The results are from a database of 85399 female patients – among the patients, 230 reported the use of SERMs Tamoxifen, Nolvadex and Faslodex. Concentrations of estradiol were determined by serum dosing that uses the chemiluminescence methodology. As cut-off point was used 356.7 pg/mL. **Results**: Of the 230 samples analyzed, 38 presented altered results. A percentage of 16.52% of patients that due to the mechanism of action of these drugs should have decreased results were found with increased results, because as these drugs have chemical structures similar to estradiol, there is cross reactivity possibility and inconsistent results referring to patients with clinical history. This observation confirms the importance of knowing the medication historic of the patient. **Conclusions:** Another interesting point of the study is that 26904 patients who did not report the use of medications and among these, 858 samples showed results above normal, which suggests the need for a more detailed evaluation of the person responsible for releasing the result, because this increase can be generated by the influence of medications as well as by other factors. Therefore, SERMs may contribute falsely increasing estradiol levels.

## A-168

### Indirect estimation of reference intervals from laboratory information systems for free thyroxin (FT4) and free triiodothyronine (FT3) in Ethiopian adults

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**Aim**

To establish indirect reference intervals for free thyroxin (FT4) and free triiodothyronine (FT3) for Ethiopian adults using data from laboratory information systems collected during routine laboratory activities as an alternative to resource-intensive population based methods.

**Methods**

All results for free thyroxin (FT4, n=7774 samples) and free triiodothyronine (FT3, n=7087) that were recorded in Ethiopia Public Health Institute, Clinical Chemistry Laboratory's laboratory information system between 2013 and 2016 were included in this study. Both FT4 and FT3 were measured using the Roche Cobas e 411 Clinical chemistry analyzer. We used the Reference Limit Estimator by Arzideh et al. to establish reference intervals, which estimates the proportion of samples from healthy individuals from a mixed population containing both pathological and physiological samples using a maximum-likelihood approach.

**Results**

We calculated combined reference intervals for males and females for FT3 (2.31-4.62 pg/ml) and FT4 (0.78-1.75 ng/ml).

**Conclusion**

Using laboratory information system data is an alternative method to validate and/or establish references intervals for low-and middle-income countries were laboratories often use kit insert references intervals, which are established in western countries and do not necessarily apply to the local population.

## A-169

### Performance Evaluation of the Atellica CH Enzymatic Hemoglobin A1c Assay*

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**Background:** According to the World Health Organization, 422 million adults were living with diabetes globally in 2014, and an estimated 1.6 million deaths directly related to diabetes occurred in 2015. Eating a healthy diet, exercising regularly, and maintaining body weight are instrumental in delaying the onset of type 2 diabetes; early diagnosis is important for long-term diabetes care. One measurement of glycemic states is glycated hemoglobin (HbA1c). HbA1c is formed by a nonenzymatic Maillard reaction between glucose and the N-terminal valine of the β-chain of HbA, whereby a labile Schiff base is formed and converted into the more stable ketoamine (irreversible) via an Amadori rearrangement. A new enzymatic HbA1c Assay (A1c_E) has been developed* for the measurement of HbA1c on the Atellica® Solution Clinical Chemistry Analyzer. In the pretreatment step, the erythrocytes are lysed and the hemoglobin is oxidized to methemoglobin by reaction with sodium nitrite. In

the first step of the reaction (the Atellica A1c_E pack 1 + sample), the N-terminal fructosyl dipeptide fragment is cleaved from the hemoglobin beta chain with a protease. Concurrently, methemoglobin is converted into stable azidemethemoglobin in the presence of sodium azide, and the total hemoglobin concentration is determined by measuring the absorbance at 478/694 nm. In the second step of the reaction, fructosyl peptide oxidase (FPOX) is added to react with the fructosyl dipeptide to generate hydrogen peroxide. The hydrogen peroxide reacts with the chromagen in the presence of peroxidase to develop a color that is measured at 658/805 nm. **Methods:** Assay linearity was evaluated using Clinical and Laboratory Standards Institute (CLSI) protocol EP06-A. Precision was evaluated according to CLSI protocol EP15-A3. Two levels of a commercially available control and four whole blood pools ranging from ~4.50 to ~12.00% HbA1c were tested. Each sample was assayed five times per run, two runs per day, for 5 days. A method comparison study (n = 40 samples) was conducted between the Atellica CH A1c_E Assay and the National Glycohemoglobin Standardization Program (NGSP) secondary reference lab according to CLSI protocol EP09-A3. **Results:** The Atellica CH A1c_E Assay is linear from 3.80 to 14.00% HbA1c. Repeatability ranged from 0.29 to 0.65% CV, and within-lab precision ranged from 0.62 to 1.09% CV. The method comparison study yielded a regression equation of Atellica A1c_E Assay = 1.047 [NGSP] - 0.377% HbA1c (r = 1.00). The assay demonstrated a %TE ≤3.57 on the Atellica CH Analyzer. **Conclusions:** The A1c_E Assay on the Atellica CH Analyzer from Siemens Healthineers demonstrates acceptable precision and correlation with the NGSP reference method. *Under development. The performance characteristics of this device have not been established. Not available for sale. Product availability will vary from country to country and will be subject to varying regulatory requirements.

## A-170

### Percentages of Hypo and Hyperthyroidism, findings of a large laboratory in the city of São Paulo.

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**Background:** Thyroid dysfunctions are very common in the world and primary care requires public policies with fast diagnostic and sensitive and specific tests as well as effective therapies for the treatment of their most prevalent disorders. From laboratory tests, thyroid stimulating hormone (TSH) dosing helps to diagnose a condition known as "subclinical hypothyroidism," which usually does not cause signs or symptoms, but hormone levels of TSH present at increased rates. The TSH test is also useful in the initial assessment of thyroid function and preferably use second or third generation assays that provide better diagnostic certainty over other traditional methods. TSH measurement is the most reliable test to diagnose the primary forms of hypothyroidism and hyperthyroidism, especially on an outpatient basis and in public health campaigns. Where possible, determination of the thyroxine free fraction (T4L) should be requested, since abnormalities in thyroid hormone-carrying proteins (secondary to the use of medications or certain clinical conditions) may alter the total concentration of T4 or T3. The ultra-sensitive TSH dosage (sensitivity 0.02 mIU / L) is the test of choice for the diagnosis of frank or subclinical hyperthyroidism. Excess thyroid hormones from any cause (except in rare cases of increased TSH) will result in suppressed TSH (usually <0.1 mIU / L); serum concentrations of free T4 will usually be elevated; in the absence of elevation of free T4 and presence of suppressed TSH, free T3 should be titrated (sometimes this is the first hormone to rise in both Graves' disease and toxic nodular goiter. OBJECTIVE-The authors aimed to analyze the results of TSH and T4L from a database of a large laboratory that serves several health units in Sao Paulo- Brazil. METHODS-The study was retrospective and observational for the period from 01/01/2012 to 12/31/2016, the results evaluated were patients of both sexes, above 18 years. The methodology used in the quantitative measurements of TSH and T4L in this period was a chemiluminescent assay, performed in an automated apparatus Architect i2000SR Immunoassay Analyzer - Abbott® Laboratories. RESULTS-The total number of requests in the period (2012 to 2016) was 4000299, respectively, 670326 (2012), 741418 (2013), 744082 (2014), 781664 (2015), 1062809 (2016).The analysis was based on calculating the percentages of the following situations: TSH and T4L, above / below the reference limits, adult TSH = 0.34 to 5.60 uIU/mL and T4L = 0.54 to 1.60 ng/dL, where for each situation analyzed we find the following percentages respectively in the years 2012, 2013, 2014, 2015 and 2016. Normal: 88.9%, 89.3%, 90.6%, 89.6%, 82.1%; Hyperthyroidism: 7.3%, 7.1%, 6.0%, 7.2%, 7.10%; Hypothyroidism: 2.6%, 1.9%, 1.7%, 1.5%, 9.90%. CONCLUSION-Based on the data found, we can say that even with the increasing number of exams done by the laboratory, the prevalence of altered hyperthyroidism data remained constant.Increased requests suggest greater interest resulting from public awareness and campaigns.

## A-171

### Hemoglobin A1c analysis using uncentrifuged & centrifuged samples.

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**Background:** The measurement of hemoglobin A1c (HbA1c) and plasma glucose using JCA-BM6010/c analyzer can be performed in the same tube to reduce the number of sample tubes required. Therefore, the recommendation of specimen used is venous blood after centrifugation (800 g, 5 min). Occasionally, HbA1c measurement is requested without plasma glucose. The objective of this study is to compare HbA1c measurement using uncentrifuged and centrifuged samples analyzed by JCA-BM6010/c analyzer as well as compare with HbA1c analysis using Cobas c513 analyzer. **Methods:** We collected 215 patient samples that were sent to the Central Laboratory, Department of Clinical Pathology. HbA1c measurement was performed using three different methods: (i.)Roche Cobas c513 (uncentrifuged whole blood) (ii.) JCA-BM6010/c (centrifuged whole blood) (iii.)JCA-BM6010/c (uncentrifuged whole blood; 800g x 5 minute). Hemoglobin concentration analysis was performed using Sysmex XN-3000. Samples were divided into 5 subgroups according to the level of hemoglobin (Hb): (1) <7 g/dL; (2) 7-9.9 g/dL; (3) 10-12 g/dL in female or 10-13 g/dL in male; (4) 12-15 g/dL in female or 13-15 g/dL in male; (5) >15 g/dL **Result:** Median (IQR) of HbA1c value (%NGSP) were 6.2 (5.7-7.2), 6.4 (5.9-7.4), 6.3 (5.9-7.3)% in c513, centrifuged JCA-BM6010/c and uncentrifuged JCA-BM6010/c, respectively. (P <0.001). Using Passing-Bablok regression analysis, the comparison of HbA1c analysis between c513 and centrifuged JCA-BM6010/c yielded a slope of 1.00 (CI 0.98 to 1.00) and intercept of 0.20 (CI 0.20 to 0.35). The comparison of HbA1c analysis between c513 and uncentrifuged JCA-BM6010/c yielded a slope of 0.50 (CI 0.36 to 0.63) and intercept of 0.94 (CI0.92 to 0.96). The comparison of HbA1c analysis between centrifuged and uncentrifuged samples using JCA-BM6010/c yielded a slope of 0.16 (CI -0.10 to 0.30) and intercept of 0.96 (CI 0.94 to 1.00). **Conclusion:** Both centrifuged and uncentrifuged samples gave comparable results in the analysis of HbA1c using JCA-BM6010/c analyzer. However, HbA1c analysis using JCA-BM6010/c showed systematic difference (centrifuged samples) as well as systematic and proportional differences (uncentrifuged samples) when compared with HbA1c analysed by c513 analyzer.

## A-172

### Correlation of Thyroid Function with Biochemical Parameters And Baseline Characteristics in Obese Individuals

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**Background:** The prevalence of obesity is increasing worldwide and at the same time, the understanding of its pathogenesis and metabolic consequences is markedly advancing. Thyroid hormones (TH) play a key role in regulating energy homeostasis. Obesity has driven new interest in the relationship between thyroid hormone and weight status. **Aim:** To know the correlation of thyroid function with biochemical and baseline characteristics in obese subjects. **Method and materials:** This is a hospital based case-control study. There were 77 obese subjects (30 male and 47 female) and 50 controls (14 male and 36 female) with age and sex matched. Five ml of the venous blood was collected and kept in 12''x75'' gel tubes. Serum samples were used for biochemical parameters using (Erba Mannheim) XL- 300 Chemistry auto analyzer and thyroid function tests in CLIA. Waist and hip circumference were measured using a measuring tape. Statistical Analysis was done using SPSS version 17. Comparisons of mean values between controls and cases were done using students't' test. Pearson's bivariate correlation analysis was used to correlate variables between the controls and cases. $p < 0.05$ was considered to be statistically significant. **Results:** The age of control and obese subject (mean±SD)were33.08±11.02 and 35.88±9.37 respectively. Demographic parameter BMI, W/H Ratios and Waist circumference (mean±SD) in control and obese subjects were 21.90 ± 1.39, 0.86±0.08, 76.00±6.94 and 31.50 ± 5.09, 0.94±0.07, 96.24±17.34 respectively. The thyroid function test and biochemical parameters in control and obese subjects FT3, FT4, TSH, FBS, TC, TG, LDL-C were insignificant and HDL-C was significant. In this study, 5% obese subjects were having a thyroid disorder, out of which 75% were of subclinical hypothyroidism and 25% with primary hypothyroidism. Pearson correlation analysis between serum FT3, FT4, and TSH with respect to baseline characteristics of the study subjects reveal positive significant correlation ($p<0.05$) between FT4

with WHR in obese subjects and insignificant correlation (p>0.05) between other baseline characteristics. Similarly, Pearson correlation analysis between serum FT3, FT4 and TSH with respect to biochemical parameters of the study subjects showed positive significant correlation (p<0.05) between FT4 with TC and LDL-C in obese subjects and insignificant correlation (p>0.05) between other biochemical parameters. **Conclusion:** Our results showed thyroid disorder in an obese subject is hypothyroidism and FT4 have a positive significant correlation with waist/hip ratio, TC, and LDL-C. **Key words:** Thyroid Hormones, Waist and Hip Ratios, Obese**,** Baseline characteristics

## A-173

### Assessment of The Effects of Gestational Diabetes on Some Anthropometric Indices.

A. M. Fisayo. *Ekiti State University, Ado - Ekiti, Nigeria*

**Background:** Gestational diabetes mellitus (GDM), a type of DM is a condition in which women develop DM during pregnancy. GDM is a threat to pregnant women because of its short and long term risks for them and their neonates. Ability to properly prevent or manage this state depends on identification of markers. This study was designed to access the effect of gestational diabetes on blood glucose and some anthropometric markers. **Methods:** The research subjects were selected from people in Ado-Ekiti metropolis of Ekiti State, Nigeria. Group 1 was made up of fifty (50) gestational diabetic women (GDM); group 2 fifty (50) normal pregnant women (NP); group 3 fifty 50 diabetic non-pregnant women (DM) and group 4 was made up of 50 non-diabetic non-pregnant women (ND-NP). Data for anthropometric measurement and blood fasting sugar were determined using standard methods. The data collected from the results were analysed using one - way Analysis of Variance (ANOVA) followed by post-hoc Duncan test, and expressed as mean ± standard deviation(SD) with P value less than 0.05 (p<0.05) considered to be statistically significant. **Results:** The results showed increased BMI in $kgm^{-2}$ in GDM (32.38±4.25) and diabetic women (DM) (31.95±12.48) compared to normal pregnant (27.85±8.58) and non-diabetic non-pregnant women (25.24±3.30). The gestational age (in weeks) of GDM (17.76±5.46) and normal pregnant (NP) women (17.62±3.33) showed no significant difference (p>0.05). There was also a significant increase (p<0.05) in FBS (mmol/l) of GDM (6.10±1.49) and diabetic patient (12.16±6.86) compared to normal pregnant (3.74±0.66) to non-diabetic non-pregnant women (4.23±0.60). **Conclusion:** It can be concluded from this study that monitoring anthropometric indices in the gestational period may serve as a means of detecting and managing gestational DM

## A-174

### Investigating Macroprolactin in a tertiary care hospital

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Background: Prolactin (PRL) circulates as a heterogeneous mix of monomeric PRL (85%) and larger molecular weight forms termed macroprolactin (macroPRL). MacroPRL is under-recognised and may cause mis-diagnosis, unnecessary investigation and inappropriate treatment of hyper-prolactinemia. The prevalence of macroPRL in hyper-prolactinemia has been variously reported as between 4.0 to 46%. All available PRL assays cross-react with macroPRL. Re-assay of such sera after polyethylene glycol (PEG) precipitation to deplete macroPRL is advised but not universally implemented in all laboratories . We investigated the prevalence of macroPRL in our immunoassay section in a 1000-bed tertiary care teaching hospital. Methods: Prolactin is performed on the Abbott Architect i2000SR immunoassay analyzer in our department. Patient requests for serum prolactin (n = 616) were studied over a 9 month period. Consecutive samples with PRL >600 mIU/L (n=100) were stored at -20°C and re-tested after treatment with equal volume of 25% PEG6000 at room temperature for 10 mins. Following centrifugation (20000g for 2 minutes) PRL was measured in the supernatant. Samples with PEG-precipitated PRL of <40%, 40-60%, >60% were considered as negative, borderline or positive for macroPRL respectively. We also compared the PRL data with those performed similarly on another immunoassay platform (Roche Cobas e602 analyzer) . Results: Pre-PEG PRL ranged from 605-18326 mIU/L (median 1178) for Architect PRL and 316-24159 (median 1289) for Cobas PRL. The post-PEG PRL ranged from 91-14346 mIU/L (median 742) ) for Architect PRL and 105-19336 (median 1608) for Cobas PRL.. The Architect PRL identified 18 subjects (13 men) as macroPRL, 11 borderline and 71 negative while the Cobas PRL classified 12 patients (8 men) as macroPRL, 5 borderline and 83 as negative. For the Architect macroPRL pre-PEG PRL ranged from 635-1523 mIU/L and declined to 91-356 mIU/L after PEG treatment while the corresponding vales for Cobas macroPRL was 401-1327 mIU/L and 105-462 mIU/L respectively. All 12 macroPRL classified by Cobas were also identified as such by Architect. Passing-Bablok regression analyses showed closer agreement between Architect and Cobas post-PEG PRL values (regression equation: Cobas = 1.373108 Architect + 0.149142, Spearman correlation coefficient of 0.972) than pre-PEG PRL values (regression equation: Cobas = 1.399187 Architect - 197.355195, Spearman correlation coefficient of 0.899). Conclusion: There was greater impact of macroPRL on hyper-prolactinemia with the Architect assay than the Cobas. However, there was 100% concordance between Cobas identified macroPRL and Architect macroPRL. The preliminary prevalence of macroPRL in our study (18% with Architect PRL and 12% with Cobas PRL) is not inconsequential. It is prudent for clinical laboratories to provide value and accurate results. A reflex investigation for macroPRL in all cases of hyper-prolactinemia is such an initiative.

## A-175

### Vitamin-D diminishes high platelet aggregation found in patients with Type 2 Diabetes Mellitus

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**Background:** Type 2 diabetes mellitus (T2DM) is associated with increased risk for atherosclerotic diseases. Platelet activation is found in inflammatory conditions and implicated in the pathogenesis of T2DM and atherosclerosis, which are also associated with Vitamin-D deficiency. The aim of this study was to investigate the relation between platelet aggregation, Vitamin-D and HbA1c among healthy individuals and those with T2DM. The direct effect of Vitamin-D 1-25 (calcitriol) on platelet aggregation was also investigated. **Methods:** Platelet aggregation was examined with and without calcitriol pre-treatment, using collagen or adenosine diphosphate (ADP) as agonists in study groups: A. normoglycemic: HbA1c<5.7; B. Pre-DM: 5.7%≥HbA1c≤6.4%; C. DM and aspirin therapy: HbA1c>6.4(+)Asp.; and D. DM not on aspirin therapy: HbA1c>6.4(-)Asp. **Results:** Platelet aggregation was higher in DM(-)Asp compared to normoglycemic and DM(+)Asp, and higher, but not significant compared to pre-DM. The study population exhibited negative correlation between HbA1c and Vitamin-D25 serum concentration. Excluding DM(+)Asp, aggregation induced by collagen was significantly higher in patients with insufficient (<76nmol/L) Vitamin-D25 compared to sufficient (≥76nmol/L) Vitamin-D25. Negative correlation was found between Vitamin-D25 serum concentrations and collagen-induced aggregation. In the DM(-)Asp, collagen-induced aggregation decreased after calcitriol treatment. Calcitriol reduced ADP-induced aggregation in control and DM(+)Asp groups. **Conclusion:** High platelet aggregation is associated with high HbA1c and low Vitamin-D25 levels. This elevated aggregation could be regulated by a novel, direct effect of calcitriol, indicating a beneficial effect of Vitamin-D on atherosclerosis and on vascular complications related to diabetes. We suggest a non-genomic mechanism for the Vitamin-D/Vitamin-D receptor (VDR) pathway.

## A-176

### Polymorphisms of LEP, LEPR, DRD2, HTR2A and HTR2C genes and risperidone- or clozapine-induced hyperglycemia

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**Background**: To determine whether the genetic polymorphisms, LEP promoter 2548G/A (*LEP*), LEPR c.668 (*LEPR*), dopamine D2 Tag-SNP (*DRD2*), serotonin 5-HT2A (*HTR2A*) and 5-HT2C (*HTR2C*), associate with risperidone- or clozapine-induced hyperglycemia in Thai adult psychotic patients. **Methods**: In this cross-sectional analysis, blood samples were obtained from 180 Thai psychotic patients treated with a risperidone (n=130) or clozapine-based (n=50) regimen. Blood samples were genotyped for the above-mentioned polymorphisms by using the TaqMan assay (Roche Diagnostics, USA); they were also analyzed for glucose, lipid profile; i.e. total cholesterol, triglycerides, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol, and hormones; i.e. adiponectin, leptin, insulin and prolactin. Differences among groups were analyzed using the $\chi^2$ test, Mann-Whitney U test or *t* test where appropriate. To determine the associations between the genetic factors as well as clinical risk factors with the hy-

perglycemia, a backward, stepwise multivariable logistic regression model was used. **Results:** The prevalence of hyperglycemia was greater among patients receiving clozapine (64.0%) than risperidone (30.8%). Metabolic biomarker results were similar in the two subject groups, except that the clozapine group showed higher fasting glucose and lower prolactin. Among candidate genes, only *LEP* 2548G/A polymorphism demonstrated significant association with the hyperglycemia ($\chi^2 = 9.879$, $p = 0.008$) in risperidone-treated patients; those with AA genotype had the highest risk (41.1%), followed by AG (20.1%) and GG (0%) genotypes. Among clozapine-treated patients, the study genes and hyperglycemia were not associated. Using binary logistic regression, *LEP* 2548G/A gene demonstrated the significant association with hyperglycemia, independent of BMI in patients on risperidone; the odds ratio (95% confident interval) was 0.314 (0.138-0.715), $p = 0.006$. By contrast, none of the polymorphisms, except for BMI significantly associated with hyperglycemia in patients on clozapine. **Conclusions:** The risk of hyperglycemia was associated with *LEP* 2548G/A polymorphisms among Thai adults receiving risperidone, but not those receiving clozapine. Polymorphism of *LEP* 2548G/A may affect the risperidone-induced glycemic dysregulation in Thai patients. The other polymorphisms under study did not appear to have any impact on the risk of hyperglycemia. Understanding the mechanisms and risks for hyperglycemia provides an opportunity to prevent impaired glucose metabolism in patients taking risperidone or clozapine.

## A-177

### Derivation of truer metrics of long term patient variation of three contemporary hemoglobin A1c assays demonstrates both borderline and highly acceptable analytical performance

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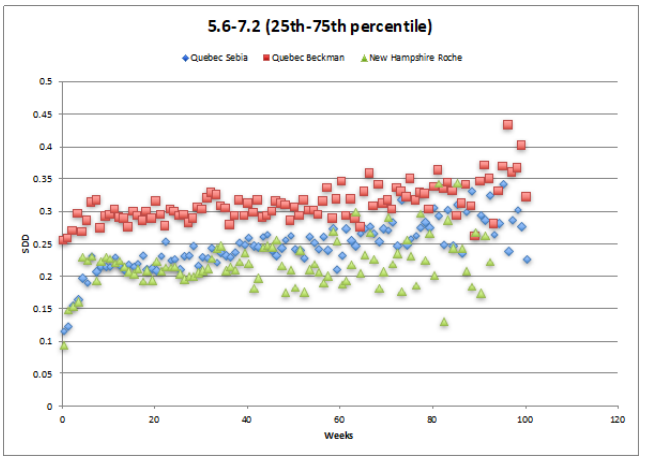
**Introduction:** Previously, we demonstrated that the statistical analysis of sequential intra-patient data can yield realistic measures of patient biologic and analytic variation. We have refined this analysis to determine long term (LT) intra-patient variation. To accomplish this, we determined all possible intrapatient pairs reported by the laboratory for several years and sorted these pairs by time between their sequential assays. The standard deviation of duplicates (SDD) was determined and charted for each time interval. We apply this analysis to three A1c assays. **Methods and Materials:** Patient HbA1c data were obtained from a Quebec laboratory replacing it's Beckman Coulter Synchron DxC®immunoassay ( 35,000 A1c from 15,000 patients) with Capillarys 2 Flex Piercing® (C2FP), ( 40,000, from 19,000 patients) and a New Hampshire laboratory operating the Tinia Quant Gen III, Cobas 8000, c502. AND Cobas 6000, c501 (121,000 HbA1c from 53,000 patients). We generated graphs of the LT intra-patient SDD of the individual methods for 3 patient subpopulations: low normal HbA1c, adequate glycemic control and poor control. **Results:** The Figure shows the LT SDD for the 25th to 75th percentile. The Beckman assay demonstrates the highest variation which is not evident in the Sebia assay which overlaps the Roche SDD. For the graphs of the other two populations, the low normal and the poor diabetes control patients, the SDDs overlap. **Discussion:** Essentially, the same patient population was sampled and assayed with the Beckman and Sebia assays. The Beckman assay obviously exhibits excess analytic variation. Sources of this variation include between instrument and between reagent lot variation. The magnitude of this increase in variation is roughly 0.1 divided by 6.5 or about 1.5%. For decades, we have maintained that the CV of HbA1c should be 2 to 3%. Future evaluations of the performance of HbA1c assays should include LT estimates of variation derived from stratified patient data.




## A-178

### Serum Testosterone as a Severity Marker among Patients with Coronary Artery Disease

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**Background:** Male sex has been considered as an independent risk factor for cardiovascular disease (CVD) since many years. But recent studies have shown controversial results. The purpose of this study is to know the relation between testosterone and degree of severity of coronary artery stenosis in men diagnosed with coronary artery disease (CAD) via angiography. **Methods:** In this cross sectional observational study, 102 men were grouped into three categories according to the testosterone level tertiles. The inclusion criteria were male patients with CAD (angiographically proven), admitted in general ward of Manmohan Cardiothoracic Vascular and Transplant Center, Nepal, who provided written consent for the study. The exclusion criteria were- Patient refusal to participate in the study; Patients on any medication affecting sex hormone level like anticonvulsant and antithyroid drugs; Patient with carcinoma of prostrate or prostatectomy; Patient with major organ failure (respiratory/renal/liver); History of recent surgery or major trauma (within 3 months); Previous angioplasty.A brief medical history and morning fasting sample were obtained from each patient and blood sugar, total testosterone (TT) and lipid profile, SHBG were measured. Blood sugar and lipid profile were by using fully automated analyzer, BT 3000, Italy. Total testosterone (TT) was measured by enhanced chemiluminescent immunoanalyzer (ECI) and sex hormone binding globulin (SHBG) by ELISA kit. Free testosterone (FT) and bioavailable testosterone (BT) were calculated and for severity of coronary stenosis gensini score was used. The relationships were assessed using chi-square test, one way analysis of variance (ANOVA) and Pearson's Correlation. **Results:** Of the total 102 patients (mean age 62 years), majority of them (41.2%) had triple vessel disease.TT, SHBG, FT and BT were 346.1 ± 176.6 ng/dl, 44.5 ± 21.7 nmol/L, 0.2 ± 0.2 nmol/L and 5.0 ± 3.5 nmol/L respectively. Various CVD risk factors had no significant correlation with testosterone. Though negatively correlated, no significant association was found between gensini score and FT and BT (r=-0.054, p-value = 0.590 and r = -0.051, p-value = 0.617 respectively). Similar results were obtained when number of vessels involved and TT, FT and BT were compared. However, the number of diabetic patients gradually decreased with the increasing value of TT in the three tertile group (p-value = 0.040). **Conclusion:** Our study suggests that low testosterone is associated with risk of diabetes mellitus. However, it cannot strongly agree or disagree with negative relation between TT and CAD, and thus warrants further investigations which may include but not limited to use of measured value of FT and BT.

## A-179

### Immunoassay harmonization (or the lack of it). The PTH assay example.

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**Background:** Capture and signal antibodies and standard/calibrator characterize immunometric assay design. Whatever combination, for harmonized assays, it is expected that different reagents give comparable results for the same patient sample. Two key components for immunoassay harmonization are the standard/reference system (that should be referred to an international reference preparation, if there is one), and antibodies combination that can recognize different epitopes. Harmonization, or lack of it, is visualized in external quality assessment schemes (EQAS). A clear example of this issue is plasma PTH measurement. Intact parathyroid hormone (PTH) is an 84-amino acid peptide, being the N terminal residue biologically active. Both plasma PTH 1-84 and PTH 1-34 disappear in few minutes. The C-terminal portion is more stable, particularly in patients with chronic renal failure (CRF), one of the clinical conditions in which PTH measurement is required. There are some other related proteins too, as PTH 7-84 and smaller fragments. Between-method differences are observed in EQAS, patients´ results show similar differences. **Methods:** To analyse the possible reasons of this lack of harmonization, components of the most used PTH intact and 1-84 PTH assays in the EQAS Buenos Aires (ProgBA) were compared. Data were taken from inserts provided by each IVD manufacturer. **Results:** a) *PTH reference preparations:* ABBOTT 79/500; CENTAUR 79/500 (73 % mean WHO standard recovery); ROCHE 95/646; IMMULITE (traceable to an internal standard); LIAISON not stated; BECKMAN 79/500 (average WHO recovery 57 % and 53 % for Routine and Intraoperative Modes). *b) Immunoassay design:* Capture and signal antibodies were different for each company, their selectivity was to peptides 1-84, 1-34, 1-37, 38-84, 39-84, 44-84, depending IVD manufacturer. ROCHE and LIAISON developed assays that claim to recognize the complete molecule, 1-84. Different selectivity against PTH fragment 7-84 was stated: 48.3 % for IMMULITE, 72 % for BECKMAN, 52 % LIAISON intact, LIAISON 1-84 0%, others not stated *c) EQAS results:* for a sample from CRF patients, medians in pg/mL were: ARCHITECT: 606, BECKMAN: 350, CENTAUR: 436, IMMULITE: 484, ROCHE INTACT: 356, LIAISON 1-84: 166, ROCHE: 1-84 187. The standards utilized by different IVD manufacturers are not the same, there are two reference preparations, 79/500 and 95/646, with even different assay recovery. Some methods do not state calibration to an IRP. When immunometric assay design is analyzed, it can be noticed that selectivity of antibodies used is quite different for each kit, detecting related peptides in different proportions. 1-84 methods give lower results as expected, but they don´t seem to be harmonized. These differences are shown in EQAS results, stressing the importance of EQAS in methodologies´ follow-up. **Conclusion:** If immunometric assays are calibrated against different preparations IRPs, if available, and antibodies in immunoassay designs recognize different fragments, active or not, it is impossible to achieve harmonization. In order to produce clinically useful and comparable patient results it is crucial that IVD industry agrees in selectivity and calibration to expand the traceability chain to higher order.

## A-180

### Evaluation Of Beta hydroxybutyrate (STANBIO Laboratory) Reagent On Beckman Coulter AU5800 Chemistry Automated Analyser

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**Introduction**
Ketosis is a common feature in acutely ill patient, patient with acute alcohol abuse, Diabetes Mellitus and starvation. Measurement of β-hydroxybutyrate (BOHB) rapidly and reliably is critical for diagnosis and management of ketosis crisis, hence we evaluated and compared 2 different BOHB kits on the Beckman Coulter AU5822 automated platform to determine their ease of use and turn-around-time (TAT)
**Method**
STANBIO Laboratory β-hydroxybutyrate LiquiColor reagent (STANBIO) and RANBUT (Randox Laboratories) reagent are both an end-point enzymatic assay. They were evaluated for correlation, precision, linearity, detection of limit and dilution verification (performed on-board of analyser).
**Results**
26 patient samples with concentration ranging from 0.15 mmol/L to 2.85 mmol/L correlates well with y=1.0183x + 0.0386, r = 0.9973. Absolute difference varied between -0.10 mmol/L to 0.22 mmol/L with percentage difference between -14.3% to 22.2 %. Slightly positive bias was observed across analytical range (up to 3.20 mmol/L) especially at the lower concentration. At lower concentration from 0.15 mmol/L to 0.45 mmol/L, correlation is y = 1.1053x-0.0041, r = 0.986, n = 11. Due to differences in upper analytical range (STANBIO at 4.50 mmol/L as compare 3.20 mmol/L for RANBUT), 6 additional samples were included. Correlation fairs even better with y = 1.0057x - 0.0032, r = 0.9993, n = 32 for BOHB concentration up to 4.10 mmol/L. Absolute difference ranges from -0.01 mmol/L to 0.22 mmol/L with percentage difference -3.2% to 8.3%. Total imprecision was 0.3% to 3.0% CV for concentrations ranging from 0.17 mmol/L to 3.52 mmol/L whilst higher CV of 1.6% to 3.4% for concentration between 0.26 mmol/L to 2.99 mmol/L for RANBUT. Linearity is within ± 10.0% for concentration between 0.18 mmol/L to 4.15 mmol/L while RANBUT fairs better with ± 5.0% between 0.25 mmol/L to 2.86 mmol/L. Lower detection limit for both STANBIO and RANBUT is the same at 0.02 mmol/L. With AU5800 onboard auto-dilution 1:2 ratio using deionized water shows 95.2 % to 102.7 % recovery with BOHB concentration up to 6.81 mmol/L and similarly RANBUT reagent recovers 92.6 % to 107.9 % with concentration up to 3.70 mmol/L. Assay time for both reagents is 10 minutes and average TAT is between 30 minutes with Laboratory Automated System. With RANBUT reagent, on average up to 15% of patient samples requires further dilution. Occasionally, assay exhibits kinetic error due to reaction instability although BOHB concentration is well within claimed analytical range. Up to 60 minutes is need for either a neat sample re-run or further automated and sometimes manual dilution. These additional interventions compromised the desired TAT significantly. However, these phenomena were not seen with STANBIO reagent.
**Conclusion**
STANBIO assay demonstrates good analytical performance and precision on Beckman Coulter AU5800 analyser. In summary, STANBIO reagent is more suitable for automated instrument design for Laboratory Automated System for fast turn- around-time resulting

## A-181

### Are the immunoturbidimetry and HPLC techniques interchangeable in the determination of glycosilated hemoglobin?

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**Background:** Diabetes mellitus (DM) is a disease characterized by an alteration in the metabolism of carbohydrates, defined by chronic hyperglycemia. HbA1c is a commonly used tool for the management and adjusting the treatment of diabetic patient's. The American Diabetes Association (ADA), the European Association for the Study of Diabetes and the International Diabetes Federation, after reviewing existing evidence, recommend it as a diagnostic test for DM when its values are greater than or equal to 6.5%.
**Methods:** The aim of this study is to compare 2 automated methods to measure HbA1c based on different principles of measurement HPLC (AKRAY HA 8180V, Menarini Diagnostics) and immunoturbidimetry (Tina-quant Hemoglobin A1c Gen.3, Cobas 311, Roche Diagnostics), evaluating the correlation Between both. We worked by verifying the correct quality requirements for both measuring instruments. 450 samples of whole blood (EDTA) were analyzed by both analytical systems
**Results:** HbA1c values were obtained between 4.4% and 13.3% (median = 6.4%) by HPLC and between 4.5% and 12.4% (median = 6.4%) by immunoturbidimetry. The Rho correlation coefficient of Spearman was 0.99 ($p < 0.0001$). Using the Bland and Altman test, we obtained an average of the differences between both methods of 0.04% and the regression of Passing and Bablot was HPLC = 0 + 1 x Immunoturbidimetry. These results corroborate results obtained previously in our laboratory with a smaller number of samples
**Conclusion:** It should be considered that changes will be made in the therapeutic regimens guided by the HbA1c level of the patient and the sequential changes of their measurements, whether or not they know the analytical performance of the method: adequate or not. This condition that undoubtedly, must be ensured by the biochemical professional. The clinical laboratory has a great responsibility in the choice of the analytical method to quantify HbA1c before the wide range of methodological possibilities offered by the in vitro diagnosis. Although the complexity of the laboratory is one of the factor that will influence this choice, it's neccesary to ensure the use of reliable, high quality tests that meet the stipulated analytical requirements, because it will directly impact the quality and clinical utility of the laboratory. result issued. At the beginning, the determination of HbA1c showed great variability between the different methods and laboratories. Currently, analytical methods can be considered "interchangeable" The correlation between both methods of measurement was very high and the average of the differences between both methods was negligible. Both methods can be interchangeable. The HPLC includes the chromatogram, with which most of the variant hemoglobins can be separated and identified, and can even show silent hemoglobinopathies. However it is an instrument of exclusive use, and other

determinations can not be made simultaneously. Acording with the bibliography the advantages of the immunoturbidimetric method are optimization of processing time of HbA1c tests and a reduction in the unit cost per test. Although HPLC is the reference technique, immunoturbidimetry is a reliable method for measuring HBA1c.

## A-182

### Effect of Sample Storage Conditions on Vitamin D Metabolites

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**Background:** Methods for measuring various vitamin D metabolites are increasingly used to investigate vitamin D metabolism and its clinical associations. However, the stability of these newly measured metabolites are not well known therefore we aimed to determine the stability of vitamin D metabolites, namely 25(OH)$D_3$, 1,25$(OH)_2D_3$, 24R,25$(OH)_2D_3$, 25(OH)D2, and 3-epi-25(OH)$D_3$ in plasma samples under different standing/storage conditions. **Methods:** Blood specimens were collected from volunteers (n=20) into K2EDTA tubes (Becton Dickinson, NJ, USA), centrifugated, aliquoted, and analyzed immediately to represent baseline values. Samples were kept at +25°C and +4ºC for short-term storage (24 and 72 hours), -20ºC for mid/long-term storage (10 and 30 days) and -80ºC for long-term storage (30 days), all in dark and analyzed with high-performance liquid chromatography tandem mass spectrometry (LC/MSMS-8050, Shimadzu Co., Kyoto, Japan). Additionally, a group of derivatized extracts of the samples were kept at +4ºC and analyzed on the 5th day, to determine on-board stability. The results obtained under different conditions were compared to baseline values and relative bias percentages (RBP) were calculated. Medians of the RBP of each group were compared to calculated acceptable change limits (ACL=$\sqrt{2}\cdot Z\cdot$CVa; Z=1.96 which is determined by the 95% confidence interval value, Cva is the analytical imprecision calculated from quality control materials at concentrations similar to median analyte concentrations of volunteers). **Results:** The RBP of all metabolites did not exceed the ACL values in any of the tested groups. Plasma samples were found to be stable under the tested durations and temperatures (Table 1). Table 1. Effect of sample storage conditions on vitamin D metabolites (CVa, analytical imprecision; ACL, acceptable change limits; RBP, medians of the relative bias percentages; * derivatized samples)

| Analytes | Median | CVa (%) | ACL (%) | RBP (%)+ 25°C, 24h | RBP (%)+ 25°C, 72h | RBP (%)+ 4°C, 24h | RBP (%)+ 4°C, 72h | RBP (%)- 20°C, 10d | RBP (%)- 20°C, 30d | RBP (%)- 80°C, 30d | *RBP (%)+ 4°C, 5d |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **25 (OH) $D_3$** | 11,0 ng/mL | 9,3 | 25,8 | 11,8 | 2,8 | -0,9 | 8,1 | 4,1 | 3,7 | -4,4 | -0,4 |
| **1,25 $(OH)_2$ $D_3$** | 33,1 pg/mL | 9,1 | 25,2 | 4,4 | 6,1 | -19,1 | 12,7 | -15,6 | 10,9 | -2,4 | 9,4 |
| **24R,25 $(OH)_2$ $D_3$** | 0,6 ng/mL | 7,6 | 21,1 | -1,2 | 5,7 | 5,0 | 12,5 | 5,9 | -7,0 | -7,2 | 11,0 |
| **25 (OH) $D_2$** | 0,4 ng/mL | 5,8 | 16,1 | -4,2 | -0,1 | -1,9 | 11,3 | -1,4 | -4,6 | -8,0 | 11,8 |
| **3-Epi-25 (OH) $D_3$** | 0,5 ng/mL | 7,4 | 20,5 | 9,1 | 0,5 | -6,1 | -3,4 | -5,4 | -3,6 | -8,7 | -18,6 |

**Conclusion:** Blood samples for vitamins D analyses or derivatized extracts can be processed under the tested laboratory conditions.

## A-183

### A Method for Depleting Thyroglobulin from Human Serum for Use in Performance Monitoring of an In Vitro Diagnostic Assay

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**OBJECTIVE:** Clinically relevant performance monitoring of *In Vitro* Diagnostic (IVD) assays during the development and/or manufacturing process is best achieved using specimens from human sources containing native analyte at medically relevant concentrations. Human Thyroglobulin (Tg) exists in circulation as a 660 kDa, dimeric protein, produced by the follicular cells of the thyroid and is the precursor of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Monomeric subunits can be found in circulation as well and may react with some immunoassays. Tg concentrations in normal human serum can be 10 to 20-fold higher than those observed in patients that have undergone full or partial ablation of the thyroid gland. In these patients, the Tg levels usually are very low or negative. As a result, it is necessary to deplete this analyte in an effort to create samples for use in measuring assay performance while concurrently maintaining matrix integrity. In this present study, we describe a novel method for depleting Tg from normal human serum was evaluated. **METHODS**: To maintain matrix integrity, the mass of Tg was exploited by employing size exclusion tangential flow filtration via a 300 kDa cutoff membrane. This method was designed to deplete monomeric and dimeric Tg while retaining serum proteins having a mass less than 300 kDa, e.g. immunoglobulins and albumin. **RESULTS:** A 2.2 liter pool of human serum was processed using a Millipore Pellicon-2 Mini housing equipped with a Millipore Biomax 300 kDa cutoff cassette. The apparatus was operated in accordance with the manufacturer's instructions, and the filtrate was collected. Initial and post-filtration Tg concentrations were measured using a research phase IVD assay and determined to be 20.24 ng/mL (n=1) and 0.00 ng/mL (n=10) respectively. In theory, the anticipated process efficiency for depletion should be >99%. **CONCLUSIONS:** Size exclusion tangential flow filtration is an effective means of non-specifically reducing analytes of interest from human serum.

## A-184

### Performance Evaluation of Immunoassays on the Atellica IM 1600 Analyzer at Friarage Hospital

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**Background:** At Friarage Hospital, studies were performed to assess the analytical performance of immunoassays (IM) for the Atellica® IM 1600 Analyzer with respect to verification of precision, linearity, and method comparison with Siemens Healthineers assays on the ADVIA Centaur® XP/XPT System. **Methods:** Precision verification was performed according to EP15-A3, method comparison per EP09-A3, and linearity per EP06-A. For precision verification, three or four concentration levels were used; each level of QC materials and sample pool were tested as one run per day with five replicates per run, for five days (25 total replicates per sample for each assay). Method comparison studies were performed using approximately 44 serum samples that covered each assay range, from low to high; samples fell into four assay concentration ranges). The number of levels of linearity material ranged up to nine depending on the assay; for each assay, three replicates of each sample level were assayed. **Results:** Overall within-run and total imprecision agreed with the manufacturer's claims. Within-run IM CVs ranged from 0.0% to 7.9% and total IM CVs from 1.3% to 14.6%. Linearity studies were performed for all assays. Precision and method comparison studies are summarized below.

| | | Precision | | | Method Comparison |
|---|---|---|---|---|---|
| **Atellica IM Analyzer Assay** | **Units** | **Mean conc. (low, high)** | **Within run %CV(SD) (low, high)** | **Total %CV(SD) (low, high)** | **Atellica IM Analyzer vs. ADVIA Centaur XP/ XPT System** |
| Fer | pmol/L | 14.66, 310.32 | 2.7(0.40), 3.6(11.27) | 3.9(0.57), 5.4(16.63) | * |
| VB12 | ng/L | 173.84, 722.24 | 7.9(13.71), 2.9(21.05) | 14.6(25.3), 7.5(54.19) | * |
| VitD | nmol/L | 72.81, 245.87 | 5.1(3.75), 2.6(6.40 | 7.6(5,51), 3.6(8.87) | * |
| iPTH | pg/mL | 3.99, 91.05 | 2.0(0.08), 1.5(1.36) | 2.1(0.09), 1.7(1.52 | * |
| BNP | ng/L | 43.00, 466.08 | 2.8(1.19), 1.4(25.51) | 2.9(1.23) 2.2(39.25) | * |
| PSA† | ng/mL | 0.14, 16.03 | 3.5(0.00), 1.9(0.31) | 6.0(0.01), 2.4(0.39) | y=1.02x-0.03(XP) |
| AFP† | IU/mL | 29.24, 223.77 | 3.7(1.10), 2.4(5.26) | 4.4(1.29), 4.2(9.37) | * |
| CEA | u/L | 2.53, 38.85 | 4.7(0.12), 2.2(0.84) | 6.4(0.16), 2.5(0.98) | * |
| eE2 | pmol/L | 139.40, 3594.36 | 3.7(5.22), 2.4(85.04) | 6.6(9.14), 3.5(124.1) | y=0.99x-34.3(XPT) |
| ThCG† | mIU/ mL | 5.71, 395.38 | 5.5(0.31), 2.2(8.63) | 6.6(0.37), 2.4(9.34) | * |
| PRGE | nmol/L | 3.69, 68.89 | 4.9(0.18), 2.7(1.85) | 7.5(0.28), 3.2(2.20) | y=0.999x-0.47(XPT) |
| TSTII | nmol/L | 0.72, 43.23 | 2.9(0.02), 6.2(2.70) | 5.3(0.04), 8.1(3.50) | y=0.98x+0.14 |
| TSH3UL | uIU/ mL | 0.01, 29.69 | 0.0(0.00), 1.3(0.39) | 0.0(0.00), 1.3(0.40) | * |

*Comparison not completed at this time. **Conclusions:** Overall the assays tested on the Atellica IM 1600 Analyzer demonstrated good precision and correlation to the ADVIA Centaur XP/XPT System assays. Generally, the precision results were consistent with manufacturer's claims. HKD also at nstitute of Cellular Medicine, Newcastle University, United Kingdom. Siemens Healthineers supported the study by providing systems, reagents and protocols and contributed to data analysis. † Not available for sale in the U.S. Future availability cannot be guaranteed.

## A-185

### Comparison of Sample Preparation Options for the Extraction of a Panel of Endogenous Steroids from Serum Prior to UHPLC-MS/MS Analysis

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**Background:** This work details sample preparation options for a panel of endogenous steroids from serum by LC-MS/MS. MRM transitions, chromatography and mobile phase additives were studied in positive and negative ionization modes. Emphasis was placed on the sample preparation to provide highly reproducible recoveries while minimizing matrix effects. Solid phase extraction was compared to supported liquid extraction in terms of recoveries, ion suppression, phospholipid content, calibration curve performance and overall sensitivity. **Methods:**LC-MS/MS analysis was performed using a Shimadzu Nexera UHPLC system coupled to an 8060 triple-quadrupole MS. MRM transitions were selected using the most intense precursor ions in positive and negative ionization using fast polarity switching. LC conditions were selected based on analyte retention, resolution, symmetry and MS signal to noise. Target analytes were spiked into human serum. Sample preparation strategies compared polymer-based reverse phase SPE, mixed-mode cation exchange SPE and supported liquid extraction (SLE). **Results:** Separation was achieved with a Restek Raptor Biphenyl HPLC column with a combination of ammonium fluoride in water and methanol. This provided better signal to noise ratios compared to acidic mobile additives in both ionization modes. Fast polarity switching was utilized with the 8060 mass spectrometer due to the inability to fully resolve analytes requiring opposite ionization modes. Investigation of non-specific binding effects to plastic collection plates during evaporation demonstrated minimal binding when using reconstitution solvents comprising high organic content, Sample preparation was optimized for the extraction of a range of endogenous steroids using polymer-based reversed phase and mixed-mode anion exchange SPE chemistries and supported liquid extraction (SLE). For each technique extraction methodology was optimized for the panel in the presence or absence of DHEAS. Inclusion of a more polar metabolite in a largely non-polar target analyte panel can present challenges when looking for optimum extract cleanliness. Recoveries greater than 75% were typically returned for each extraction protocol. Supported liquid extraction allowed matrix spiked with ISTD without any pre-treatment to be loading onto the sorbent, thus maximizing extraction volumes. Good analyte recoveries were returned when using various water immiscible elution solvents: DCM, MTBE, EtOAc and hexane mixtures. Final extraction was performed using 25/75 hexane/EtOAc when DHEAS was absent from the panel or 100% EtOAc when present. SPE optimization resulted in wash solvent compositions up to 40% MeOH depending on mechanism. Final elution was performed using EtOAc when DHEAS was absent from the panel or MeOH when present. Evaluation of phospholipid interference demonstrated SLE to remove the highest amount for both elution solvents. When using MeOH as an elution solvent in SPE, levels were far higher than when using a water immiscible extraction solvent such as EtOAc. Full results will be presented in the poster. **Conclusion:** This paper demonstrates a sensitive, fast polarity switching method for the analysis of multiple steroids from human serum. The optimized sample preparation protocols provide sufficient extraction recovery and extract cleanliness in order to reach low limits of quantitation. The development of multiple sample preparation strategies allows for a choice of workflow dependent on laboratory requirements.

## A-186

### Assessment of bone health status and risk factors for fracture in type 2 diabetics

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**BACKGROUND:** Diabetes mellitus has profound effects on bone health. It is associated with increased glycation of bone collagen matrix that can lead to impaired bone turnover rate, decreased bone strength, increased fragility and risk of fracture. This study was conducted to determine the risk factors for fracture in T2DM patients with a view to preventing morbidity and mortality associated with bone health. **METHODS**: This case-controlled study was conducted in Lagos between July 2016 and June 2017. Participants included 90 T2DM and 77 controls made up of men and women aged 30-79 years. Physical and biochemical parameters such as BMI, WHR, phalangeal BMD, HbA1c, CTX-1, were measured in all the participants and control at first contact. Fracture risk was assessed using phalangeal BMD and FRAX. Body mass index (BMI), WHR, CTX-1 and HbA1c were measured in participants again after six months. Comparison between T2DM and controls were done using t-test and Mann-Whitney U test. Association between BMD, FRAX, anthropometric, biochemical and clinical fracture risks were done using Spearman's correlation and Chi-square tests. The level of significance was put at 5%. **RESULTS:** Phalangeal BMD did not show significant difference between T2DM and controls (p=0.230). There was no significant difference in CTX-1 levels at first contact (p=0.117), but CTX-1 was significantly lower in T2DM than controls after six months (p=0.004). The relative risks for both hip and major osteoporotic fracture are similar in T2DM and controls. (p = 0.086 and 0.243 respectively). Also, T2DM has a higher but insignificant median FRAX 10-year predictive score for developing hip and major osteoporotic fracture than the controls (p<0.757). However, the frequency of hip and major osteoporotic fractures are higher in T2DM patients than the controls. Age and duration of diabetes strongly correlate with FRAX score. (r=0.499, p<0.001 and r=0.306, p<0.001 respectively). Other clinical risk factors such as smoking, alcohol intake, cognitive and visual impairment, diabetic medications, HbA1c levels and frequent falls did not show association with fracture risk. **CONCLUSION:** The levels of CTX-1 are impaired in T2DM but with undefined phalangeal BMD. The elevated FRAX score suggests a higher fracture risk in T2DM. Subsequently, bone assessment using the above tools should be included in the routine evaluation of T2DM to determine fracture risks and complications. Appropriate intervention for high risk patients will significantly reduce morbidity and mortality in them.

## A-187

### Metabolic and Biochemical Parameters in Patients with Skin Tags

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**Background**
Acrochordon or fibroepithelial polyp, commonly known as Skin tags (STs) are one of the most common benign skin condition, consisting of skin projecting from the surrounding skin, usually occurring on the eyelids, neck and axillae, less often on the trunk and groin. Skin rubbing, skin aging and a familial predisposition are causes for STs, while others described hormonal imbalances and hyper-insulinemia as contributing factors. Studies have found an association of STs with conditions such as obesity, diabetes mellitus and atherogenic lipid profile. Abdominal obesity and the consequent insulin resistance are said be important contributing factors for diabetes, dyslipidemia and cardiovascular disease.
**Objective**
To highlight the association of metabolic parameters (body mass index, blood pressure, waist circumference) and the biochemical parameters (lipid profile, fasting glucose, HbA1c and serum leptin) levels in Nepalese patients with STs visiting the Dermatology out patient department of Teaching Hospital, Kathmandu, Nepal.
**Methods**
This study comprised of 99(men or women) presenting to the dermatology clinic where 15 males and 35 females with STs taken as cases and 14 males and 35 females of the same age and sex with no STs were taken as controls. Metabolic parameters (body mass index, blood pressure, waist circumference) along with the Biochemical parameters (serum lipid profile, glucose, HbA1c, and serum leptin) were measured in all individuals. SPSS ver. 20.0 was used to analyze the data. Mann-Whitney U test was applied for comparison of median to see the difference between case and control group and Spearman's correlation was used to establish the association between two quantitative variables.
**Results**
Serum leptin was found to be significantly higher in both male and female patients having STs than the controls at the probability level of 0.001. Also, serum leptin is seen to increase with increasing BMI in both male and female cases and controls. In male with STs fasting blood glucose, glycosylated hemoglobin, triglyceride, systolic blood pressure and diastolic blood pressure was found higher than the individuals without STs. In female fasting blood glucose, glycosylated hemoglobin, total cholesterol, triglyceride, systolic blood pressure and diastolic blood pressure was found higher than the individuals without STs.
**Conclusion**
In the present study, there is significant association of STs with triglycerides, total cholesterol, blood pressure and serum Leptin levels. It is thus implied that skin tags may be one of the important skin markers of metabolic disorders and may attract physicians and dermatologist for further investigation as it is proved to be not just a cosmetic problem. This leads us to recommend the change of life style of patients with STs and or hyperlipidemia, as stopping active smoking and prevention of passive smoking, regular exercises, weight reduction, changing carbohydrate diets into high protein diets. Knowing that diets rich in polyunsaturated fatty acids as olive oil, omega 3, 6 and 9 fatty acids supplementation can decrease the risk of coronary atherosclerosis, we recommend their use for patients with STs and/or hyperlipidemia.

## A-188

### Vitamin D status in Bangladeshi population

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**Background:** Vitamin D deficiency is a global public-health concern. Poor Vit-D status has been observed in South Asian populations. The cultural practices, lack of scopes and food habit do not facilitate the adequate sun exposure. Deficiency of Vitamin D indicated by low serum concentration of 25 hydroxy vitamin D [25(OH) D]. The synthesis of Vit-D is stimulated by the exposure to sun light. However no information is available on Vitamin D status for the adult populations who are working as corporate officials in Bangladesh. **Methods:** A total 226 subjects were included. Vitamin D Total (25(OH)D) was assessed in a study with corporate officials of a multinational company in Bangladesh, Dhaka on October 22, 2015. In addition 120 patient samples of aged 15 to 92 yrs. from different sources and occupations were run in different time from July to October, 2015. vitamin D was assayed by Chemiluminescense Immunoassay (CLIA) using Advia Centaur XP analyzer. The Deficiency is defined by 25(OH)D less than 20 ng/mL, insufficiency by 25(OH)D 20 to 29.99 ng/mL, sufficiency by 25(OH)D 30 to 100 ng/mL and toxicity by 25(OH)D above 100 ng/mL. **Results:** Among 106 adult officials (both male and female) aged 19-58 yrs. the mean 25(OH)D of 95 was 10.58 ng/mL and other 11 was less than 4.2 ng/mL. Among the rest 120 non-corporate subjects, the mean of 25(OH)D was 14.27 ng/mL and 19 were less than 4.20 ng/mL and 2 were above 100 ng/mL. It has been observed from the study that the proportion of the total officials of 25(OH)D deficiency was 97.17% and insufficiency was 2.83%. There was not a single person of sufficient level. In the other hand 80.83% deficiency was found from non-corporate group with 11.67% insufficiency, 5.83% sufficiency and 1.67% of toxicity. **Conclusion:** Vitamin D status of Bangladeshi population was poorer than might be expected based on cultural and geographic considerations. Corporate workers are more at risk than common people. Large scale awareness program needs to be initiated to combat this major public health concern.

## A-189

### Relationship Between Hyperglycemia, Inflammation And Oxidative Stress In Type-2 Diabetic Nephropathy Subjects.

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**Background:** Oxidative stress increased in diabetes generates ROS producing inflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL-6) in renal cells, which are the factors responsible for diabetic nephropathy. The study aimed to assess the correlation of hyperglycemia in relation to antioxidant status and inflammatory markers in type-2 diabetic patients with diabetic nephropathy and compare them with diabetics without nephropathy. **Methods:** Serum levels of inflammatory markers (Interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), antioxidants [glutathione reductase (GR) and glutathione peroxidase (GPx)], plasma malondialdehyde (MDA) along with other routine biochemical parameters , fasting blood sugar (FBS), urea and creatinine levels were estimated in healthy controls (n=50), diabetic subjects without diabetic nephropathy (n=50, group 1) and with nephropathy (n=50, group 2). Comparison between the groups was done using one way ANOVA and $P<0.05$ was considered to be statistically significant and correlation was done by using SPSS version 17. **Results:** Group 2 subjects have significant increase of fasting blood sugar, serum urea , creatinine, malondialdehyde and inflammatory markers (IL-6 and Tnf-α) levels with decreasing in antioxidant GPx as compared to both group 1 and healthy controls. All the parameters are showing highly significant at $P<0.05$. Fasting blood sugar was positively correlated with MDA, serum IL-6 and Tnf-α concentrations (for group 1: $r = 0.43$, $P<0.05$; $r =0.867$, $P<0.001$; $r =0.867$, $P<0.001$ and for group 2: $r = 0.47$, $P<0.05$; $r = 0.94$, $P<0.001$; $r=0.91$, $P<0.001$; respectively). Persistent hyperglycemia levels was negatively correlated with antioxidant status i.e. GPx levels (for group 1: $r = -0.68$, $P<0.001$ and for group 2: $r = -0.74$, $P<0.001$ respectively). Serum creatinine levels were positively correlated with serum IL-6 and Tnf-α only in group 2 subjects ($r = 0.75$, $P<0.001$; $r = 0.71$, $P<0.001$; respectively). Furthermore the decreasing levels of GPx were positively correlated with serum IL-6 and Tnf-α (for group 1: $r = 0.62$, $P<0.05$; $r = 0.47$, $P<0.05$ and for group 2: $r = 0.71$, $P<0.001$; $r = 0.66$, $P<0.001$ respectively) **Conclusion:** The increased levels of inflammatory markers and increased oxidative stress as evidenced by decreased antioxidant enzymes and increased cellular peroxidation products (MDA) are associated with development of diabetic nephropathy in type 2 DM. These markers could be used to predict renal progression in long standing type 2 DM.

## A-190

### A Novel Method for Free 25 Hydroxy (25OHD) Vitamin D Measurement by LC-MS/MS: Free 25OHD Associated with PTH and Calcium Better than Total 25OHD

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**Background:** Serum 25-hydroxy vitamin D (25OHD) is widely used as a biochemical marker of vitamin D sufficiency. In circulation, 25OHD is highly lipophilic and tightly bound to vitamin D binding protein (VDBP); a smaller fraction is weakly bound to albumin and <0.1% is circulating in free form. It has been demonstrated that the majority of cells in the human body respond to the free, rather than protein-bound, form of 25OHD. Therefore, measurement of free 25OHD (F25OHD) is likely more relevant than total 25OHD (T25OHD) for assessing physiologically active vitamin D concentrations. **Methods:** We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for measurement of free 25OHD. Sample preparation was performed as follows: F25OHD (F25OHD2 and F25OHD3) were separated from the protein bound fraction using size exclusion based technique, stable isotope labeled in-

ternal standards were added, F25OHD2 and F25OHD3 were derivatized and analyzed by LC-MS/MS. The method was compared to a commercial F25OHD ELISA (Future Diagnostics, The Netherlands). Concentrations of calcium (Ca) and parathyroid hormone (PTH) were determined using Cobas 8000 analyzer (Roche Diagnostics); T25OHD (T25OHD2 and T25OHD3) was measured using LC-MS/MS. As part of the method evaluation we analyzed F25OHD and T25OHD in a set of samples from self-reported healthy adults (n=251, 122 men and 129 women; age range 20-63; PTH range 14-112 pg/mL; Ca range 8.4-10.6 mg/dL), and set of residual patient serum (RS) samples representing a wide range of PTH and Ca concentrations (n=160, 65 men and 95 women; age range 18-85; PTH range 10-2244 pg/mL; Ca range 5.2-14.6 mg/dL). **Results:** The lower limit of quantification for F25OHD2 and F25OHD3 was 0.005 ng/mL; total imprecision at concentrations >0.01 ng/mL was <15%. Reasonably good correlation ($r^2$=0.787, n=62) was observed with the F25OHD ELISA, however, concentrations averaged 6.2 times lower than by the LC-MS/MS method. One likely explanation for the lower concentrations is irreversible binding of F25OHD to the labware used in the ELISA. In samples from healthy adults, we observed a better association between PTH and F25OHD (p=0.0022) than with T25OHD (p=0.082). In the RS, we observed a statistically significant association of PTH with F25OHD (p=0.015) and T25OHD (p=0.0011). In the RS group, statistically significantly lower F25OHD and T25OHD concentrations were observed in samples with Ca concentrations below 8.4 mg/dL as compared to samples within the Ca reference interval (8.4-10.2 mg/dL). Lower concentrations of F25OHD were also observed in samples with Ca concentrations above 10.2 ng/mL (p=0.05), compared to samples with Ca concentrations within the reference interval, while no association with T25OHD was observed in this group. It is known that in individuals with hyperparathyroidism and hypercalcemia, low concentrations of 25OHD could have a protective effect to prevent further increases in Ca. **Conclusion:** Our data suggest that the presented method is specific for measurement of F25OHD. Sensitivity is sufficient for quantitative measurements of F25OHD in serum samples at concentrations expected in both health and disease. Importantly, better association was observed between Ca/PTH and F25OHD than with T25OHD, indicating that F25OHD measurements likely reflect the most physiologically relevant form of vitamin D.

## A-191

### Risk Index of Gestational Diabetes as Screening Tool to Avoid Glucose Challenge Test

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**Background:** The two steps diagnostic strategies for gestational diabetes mellitus are based on one hour glucose challenge test (GCT), used as screening tool, followed by a glucose tolerance test (GTT) when positive. The sensitivity of GCT as screening tool can be similar to other biomarkers, as fasting glucose (FG), and its reproducibility is low, especially when compared to other related biomarkers such as glycosylated hemoglobin ($HbA_{1c}$). Also pregnant women must stay at the consultation room one hour to complete the test and suffer discomfort and nausea.The aim of this study is to establish a risk index of GDM ($RI_{GDM}$) based on three biomarkers: $HbA_{1c,}$ FG and triglycerides (TG), to been used as a screening test to avoid the used of GCT. **Methods:** This was a prospective study with 507 pregnant women between the 24th and 28th weeks of gestation. All the population was submitted to GCT, and 100 g, 3 hours GTT to those with GCT ≥ 140 mg/dl. Also we determined $Hba_{1c}$ (G8® from Tosoh), FG and TG (Cobas ® 8000 from ROCHE). GDM was diagnosed following the National Diabetes Data Group criteria. A multivariate logistic regression was used to obtain the parameters of the model equation. ROC curve was plotted, area under the curve was calculated (AUC) and cut-off points were established to optimized sensitivity(S) and specificity(SP). For each cut-off we determined S, E, positive and negative predictive values (PPV, NPV), positive and negative likelihood ratios (+LR, -LR), and number and percentage of pregnant women that wouldn't need GCT. SPSS 20 was used. **Results:**

$$RI_{GDM} = 1000x[1/1+e^{(18.9-0.078FG-1.62HbA_{1c}-0.009TG)}]$$

| Utility | Low Risk | | High Risk | |
|---|---|---|---|---|
| AUC (95% CI) | 0.912 (0.847-0.978) | | | |
| Cut-off | ≤20.3 | ≤8.5 | ≥710 | ≥112 |
| S (95% CI) (%) | 95.8(79.8-99.3) | 100(86-100) | 58.3(38.8-75.5) | 12.5(4.3-31) |
| SP (95% CI) (%) | 59(54.5-63.3) | 26.4(22.6-30.5) | 95.2(92.9-96.8) | 99.4(98.2-99.8) |
| PPV (95% CI) (%) | 10.5(7.1-15.3) | 6.38(4.3-9.3) | 37.8(24.1-53.9) | 50(18.8-81.2) |
| NPV (95% CI) (%) | 99.65(98-99.9) | 100(97-100) | 97.8(96.1-98.8) | 95.8(93.6-97.2) |
| +LR (95% CI) | 2.34(2.04-2.68) | 1.359(1.29-1.43) | 12.15(7.19-20.45) | 20.83(4.24-93.5) |
| -LR (95% CI) | 0.071(0.01-0.48) | 0 | 0.44(0.27-0.70) | 0.88(0.75-1.02) |
| No GCT, N(%) | 283 (56.37) | 126 (25.1) | 37(7.37) | 6 (1.2) |
| | False Negatives | | Negative GCT | |
| N | 1 | 0 | 13 | 2 |

**Conclusion:**
By using $RI_{GDM}$ as screening prior to GCT, pregnant women can be classified at low risk of GDM, avoiding to perform up to 25% of GCT, with a S= 100%, or up to 56%, with a S= of 95.8%.

## A-192

### The correlation Regression Model between HbA1c and Glycated Albumin in Chinese population

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**Abstract:**
[**Objective**]The measurement of HbA1c(Glycosylated hemoglobin) by HPLC is affected by the amount and the quality of red blood cells and some hemoglobin diseases. It is reported that the Glycated albumin(GA) is better than HbA1c in reflecting short term mean glycemia. This research focus on the correlation of GA and HbA1c and establish the fomula to estimate HbA1c by measuring the GA value. [**Methods**]20,381 subjects were recruited, including 10215 males (47 ± 12 years old) and 10,166 females (42 ± 12 years old). Using residual analysis to delete the outliers of HbA1c and GA. when HbA1c ranged from 4.0% to 12.0%, corresponding to GA ranged from 7.5% to 45%. HbA1c values between 4.0% to 8% were divided into 8 groups: <4.5%, 4.5% -5%, 5% -5.5%,5.5% -6%, 6% -6.5%,6.5% -7%, 7% -7.5%, 7.5% -8%, respectively compared the difference of GA values corresponding to HbA1c. HbA1c values during 4.0% to 8.0%were divided into 38 groups as HbA1c increases by 0.1%. The scatter plot of GA average as X-axis and HbA1c as Y-axis, Calculate the correlation between HbA1c and GA and analysis the ratio of GA / HbA1c in each group. [**Results**]The levels of GA and HbA1c have no significant difference between male and female was shown in table.1). All the data were analyzed as scatter plots, and the equation that reflects the correlation between the GA and HbA1c was HbA1c=0.181GA + 3.489,$R^2$ = 0.299 (Figure 1). It was found that the linear relationship between GA average and HbA1c of increases by 0.1% was discontinuous at HbA1c = 6.2%( GA = 12.28%), which is a turning point( Figure 2), using this breakpoint as a boundary and do the piecewise equation (Figure 3).Before and after the breakpoint equation is : when HbA1c <6.2% or GA <12.28%, the formula is: HbA1c = 1.136GA-7.289, R2 = 0.824; when HbA1c≥6.2% or GA≥12.28%, the formula is: HbA1c = 0.252GA + 3.163, R2 = 0.948. [**Conclusion**] There is a discontinuous linear relationship between HbA1c and GA. When HbA1c is 6.2%, there is a significant turning point between GA and HbA1c. Any factors that affect the amount and quality of hemoglobin will interfere with the results of HbA1c, when HbA1c and blood glucose monitoring results can be inconsistent with the evaluation of HbA1c by measuring The GA value.

## A-193

### Analytical Determination of Testosterone in Human Serum using an Ultivo LC/TQ

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**Background:** In this research study, a robust, sensitive and relatively fast analytical method was developed for the quantitation of free testosterone in serum using an Ultivo triple quadrupole mass spectrometer LC/MS (LC/TQ). Ultivo was designed to address many challenges faced by research laboratories and this research study was conducted in order to assess how this novel triple quadrupole mass spectrometer (MS) could perform with a typical endogenous analyte of research interest. Innovative technologies within Ultivo allowed for a reduction in overall physical footprint, while generating comparable analytical performance to similar, but physically larger MS systems. Innovations such as, VacShield, Cyclone Ion Guide, Vortex Collision Cell, Virtual Pre/Post Filters and small Hyperbolic Quads supported a reduction in instrument size, yet maximized quantitative performance, instrument reliability and robustness. Furthermore, Ultivo reduces the overall frequency for system maintenance, making the system operation and maintenance manageable for non-expert MS users. MassHunter Software simplifies data acquisition, method set up, data analysis and reporting, which results in the fastest possible acquisition-to-reporting time, increasing lab productivity. Herein, this research study aims to outline typical confirmation performance of free testosterone in human serum using the Ultivo LC/TQ. Lower limits of quantitation, chromatographic precision and calibration linearity, range and accuracy will be discussed. **Methods:** Sample analysis was performed using an Agilent 1290 UHPLC/Ultivo LC/TQ with electrospray ionization (ESI) in positive mode. The chromatographic column used was a Poroshell EC C18 column (2.1 x 50 mm w/ 2.7 µm). The UHPLC mobile phases were 0.1% formic acid and 5mM ammonium acetate in water (A) and methanol (B). The total chromatography cycle time was 6 minutes. Two MRM transitions are monitored for the analyte and a single transition for the deuterated internal standard. Human serum samples (250 µL) were spiked with calibrators at various concentration levels, cold acetonitrile (500 µL) containing the deuterated internal standard was added to affect protein precipitation and centrifuged at 5000 rpm. The supernatant liquid was then further diluted (1:2) with a 50:50 methanol:water solvent mixture prior to instrument injection. **Results:** Excellent linearity and reproducibility were obtained, with a concentration range from 1.0 pg/mL to 100 ng/mL (20 fg to 2000 pg on-column) for the testosterone with a linearity coefficient of > 0.999 for three batches prepared for this research study. Precision data observed over the three batches resulted with a %RSD variation of < 12% across all calibration levels in this research study. **Conclusion:** This research project demonstrates that the performance of the Ultivo LC/TQ with the analytical methodology described herein generated excellent linearity, precision and sensitivity across the range of 1.0 pg/mL through 100 ng/mL for free testosterone in human serum within an analysis cycle time of 6 minutes. Further research is needed before implementing in a routine clinical setting. For Research Use Only. Not for use in diagnostic procedures.

## A-194

### Anti-thyroid peroxidase and anti-thyroglobulin antibodies positivity in patients with hypothyroidism - is it necessary to ask for both antibodies in the evaluation of autoimmune thyroid disease?

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**Introduction:** Thyroid is a common target for autoimmune diseases (AID). Thyroid peroxidase antibodies (TPOAb) have been involved in the tissue destructive processes associated with the hypothyroidism observed in Hashimoto's and atrophic thyroiditis. There is some debate over the clinical utility of serum thyroglobulin antibodies (TgAb) measurements, since they do not appear to be a useful diagnostic test for AITD In areas of iodide sufficiency. The isolated positivity of TgAb showed no association with hypothyroidism or TSH elevations. **Objective:** To evaluate the prevalence of TPO and Tg antibodies positivity in patients with TSH levels higher than 10 mIU/L. **Methods:** We analyzed samples of both genders ≥ 12 years from a large database of a private reference clinical laboratory, tested for TSH, TPOAb and TgAb (ECLIA, Modular, Roche) in the period from January to December 2016. All the patients had TSH levels higher than 10 mIU/L. TPOAb and TgAb values, respectively, above 34 U/mL and 115 U/mL were considered positive. **Results:** 771 patients were evaluated, 72% women, mean age 52 ± 20 years; 316 (41%) of the patients had both negative antibodies; 455 (59%) presented positive TPOAb and/or TgAb. Analyzing these 455 patients, we found that 262 (58%) showed positivity of both antibodies; 147 (32%) only positive TPOAb and 46 (10%) only positive TgAb. 409 (90%) presented positive TPOAb regardless of TgAb levels (positive or negative) and 308 (68%) presented positive TgAb regardless of TPOAb levels. **Conclusions:** We found a higher positivity of TPOAb comparing with TgAb in patients with hypothyroidism, TSH higher than 10 mIU/L. Most of the patients that were TgAb positive were also TPOAb positive. Measurement of TPOAb only, allowed the diagnosis of thyroid autoimmunity in 90% of patients, suggesting that concomitant measurement of TPOAb and TgAb may be dispensable in routine evaluation of thyroid autoimmunity.

## A-195

### Commutability of processed materials with different matrix for progesterone measurement

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**Background:** the measurement of progesterone is important to determine ovarian function and to predict early pregnancy. The results from common External Quality Assessment (EQA) program differ greatly. To better interpret the EQA results and investigate the possibility of preparing commutable materials for EQA program or preparing candidate reference materials, the present study evaluated the commutability of reference materials, EQA materials, swine sera, human serum pools prepared from patient samples and hydroxypropyl-beta-cyclodextrin aqueous solution. **Methods:** an ID/LC-MS/MS method for progesterone measurement was used as comparative method. Six immunoassays (Abbott, Beckman, Chivd, Mindray, Roche, Siemens) that were commonly used in clinical laboratories were chosen as evaluated methods. Thirty-five processed materials were tested along with forty-eight individual patient serum samples. All of the samples were measured in triplicate for the routine immunoassays. The samples were tested in order then reversed. A scatter plot was generated from patient samples, and 95% prediction intervals were calculated to evaluate the statistics commutability of the processed materials. **Results:** Ordinary linear regression (OLR) was performed and the slopes of the regression lines were 0.961~1.263 and the intercepts were -1.136~0.891. The OLR and its 95% confidence intervals demonstrated that reference materials (ERM-DA347, BCR-348R, GBW09197, GBW09198, and GBW09199) were commutable for all the six immunoassays tested. The hydroxypropyl-beta-cyclodextrin aqueous solution exhibited negative matrix effects in all immunoassays. Swine sera exhibited positive matrix effects in some immunoassays. Part of EQA materials showed positive matrix effects in some immunoassays. **Conclusion:** The reference materials and human serum pools prepared from patient samples were commutable. Non-commutability of the tested EQA materials was observed among current progesterone immunoassays, which implied that interpretation of EQA results needs consideration of the bias caused by non-commutability. Other materials such as hydroxypropyl-beta-cyclodextrin aqueous solution and swine sera were mostly non-commutable and could not be used as candidate reference materials.

## A-196

### The Circadian Rhythm of Cortisol in the saliva ofpatients with mild traumatic brain injury-Comparison with healthy controls

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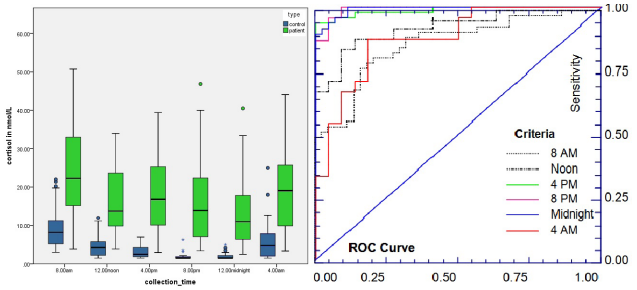
Background: Traumatic brain injury (TBI) patients represent a specific subgroup of trauma population due to activation of the hypothalamic-pituitary-adrenocortical (HPA) axis. Salivary cortisol is an accepted surrogate for serum free cortisol in the assessment of HPA-axis function. The purpose of this study was (1) to establish the feasibility of saliva cortisol measurement in mild-TBI patients, and (2) to determine the diurnal pattern of saliva cortisol in the acute phase after injury. Methods: Saliva cortisol was measured with an electroluminescent immunoassay on Cobas e-411 (Roche, Mannheim, Germany). Saliva samples were collected

and stored, according to manufacturer's specifications until tested. Saliva samples were prospectively collected from 12 mild-TBI patients (GCS=15). 11 healthy volunteers served as controls. All patients and controls were males and their mean age(±SD) was 59.4 (12.1) and 41.2 (18.9) years respectively. Collections in both patients and controls were performed on 4 consecutive days during the first week after injury, and 6 times during a day at 4AM, 8AM, Noon, 4PM, 8PM and Midnight. Results: Median saliva cortisol concentrations were significantly higher in patients versus controls at all time points ($p<0.001$) as shown at the left side of our graph. These levels remain elevated, compared to controls, during the whole follow-up period. Patients develop the expected PM versus AM decrease in cortisol concentration seen in controls ($p=0.005$). ROC-curve analysis was performed for each collection time point for patients vs. controls. Area under the curve was significantly higher ($p<0.05$) at PM versus AM collections (right side of graph). Conclusion: Our data show that in mild-TBI the HPA is activated, the diurnal pattern of saliva cortisol is maintained as seen in controls, and finally the best sampling time for saliva cortisol measurement is between 8PM and Midnight.




## A-197

### Performance of selected Fertility Panel Immunoassays (FSH, LH, Progesterone, Prolactin) on the Alinity i platform, Abbott´s Next Generation Immunochemistry System

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**Background:** Abbott offers a range of assays in the fertility panel on the Alinity i system that can assist healthcare professionals in the diagnosis and management of fertility issues by providing reliable and accurate results. Abnormal levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) may be indicative of potential gonadal failures and/or a dysfunction of the hypothalamic-pituitary axis. Abnormal progesterone levels are indicators for reproductive disorders such as infertility or pregnancy loss. Quantitation of prolactin may be useful the diagnosis of male female gonadal and pituitary dysfunctions and in the management of amenorrhea and galactorrhea. The aim of the current study is to evaluate the precision, lower limits of measurement (Limit of Blank, LoB; Limit of Detection, LoD; Limit of Quantitation, LoQ) and method comparison as key performance characteristics of the four selected assays on the newly developed Alinity i system. **Methods:** Studies to determine the lower limits of measurement and the precision of the assays on the Alinity i system were conducted based on guidance from CLSI EP17-A2 and CLSI EP05-A2, respectively. The Alinity i assays were also tested side by side with the corresponding ARCHITECT assays to generate method comparison data based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method. **Results:** The observed results for precision, lower limits of measurement and method comparison for the fertility panel assays on Alinity i are shown in the table below.

| Assay | Unit of measure | Within-Laboratory (Total) Imprecision | LoB | LoD | LoQ | Method comparison (Slope / Correlation) |
|---|---|---|---|---|---|---|
| FSH | mIU/mL (IU/L) | 1.9 - 2.7 %CV | 0.01 | 0.02 | 0.11 | 0.98 / 1.00 |
| LH | mIU/mL (IU/L) | 2.8 - 4.7 %CV | 0.02 | 0.04 | 0.12 | 0.94 / 1.00 |
| Progesterone | ng/mL (nmol/L) | 3.1 - 6.1 %CV (3.0 - 5.8 %CV) | 0.1 (0.3) | 0.2 (0.6) | 0.5 (1.6) | 0.95 / 0.99 |
| Prolactin | ng/mL (mIU/L) | 2.1 - 2.8 %CV (2.1 - 2.8 %CV) | 0.45 (9.45) | 0.47 (9.87) | 0.79 (16.59) | 1.03 / 0.99 |

**Conclusion:** The selected assays demonstrated satisfactory performance in terms of precision and lower limits of measurement on the Alinity i system. Method comparison data showed very good correlation between the Alinity i assay and the respective ARCHITECT assay.

## A-198

### Thyroid Auto-Antibodies - Impact of Change in Assay Methodology on Thyroid Testing

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**Background:** Autoimmune thyroid disease (AITD) causes cellular damage and alters thyroid gland function by humoral and cell-mediated mechanisms. AITD, commonly Graves' Disease and Hashimoto's Thyroiditis, is usually accompanied by the presence of thyroid autoantibodies such as anti-thyroglobulin antibody (TgAb), anti-thyroperoxidase antibody (TPOAb) and anti-thyroid-stimulating-hormone-receptor antibody (TRAb). Assays for thyroid autoantibodies suffer from analytical specificity and standardisation limitations, resulting in wide differences in test results and reference limits. Our study evaluated the Roche TgAb, TPOAb and TRAb immunoassays on the Roche Cobas e602 as an alternative to the Brahms Compact Plus immunoassays (TgAb, TPOAb) and the Brahms TRAK radio-receptor assay (TRAb). We also examined the reference intervals of these autoantibodies in local healthy volunteers. **Methods:** Performance validation parameters of the Roche assays included assay imprecision, lower limit of detection, linearity and carry-over. Method correlations between Cobas e602 assays and Brahms Kryptor Compact Plus (TgAb,TPOAb) / Brahms TRAK radio-receptor assay (TRAb) were tested using patient serum samples (n=130-190). Inter-assay concordance between Roche and Brahms were evaluated using the respective manufacturers' cutoffs. Reference intervals for the thyroid autoantibodies were assessed on the Roche assays in metabolically stable local healthy volunteers, using screening criteria of serum thyroid stimulating hormone (TSH) levels between 0.5-2.0 mIU/L, no personal or family history of thyroid diseases and absence of non-thyroid autoimmune diseases. **Results:** Within-run and total imprecision for the Roche thyroid autoantibody assays were determined to be ≤7.9%. All 3 assays demonstrated linearity across the manufacturer's analytical measurement range (recoveries: 96 to 125%). Lower limits of detectable concentrations of TgAb (2.2 IU/mL), TPOAb (2.3 IU/mL), TRAb (0.2 IU/L) agreed with manufacturer's claims. Results of carry-over studies were insignificant. Method correlation with the Brahms assays revealed Passing Bablok regression slopes of 2.87, 0.20, 0.87 and intercepts of -68.1, +7.1, -0.03; mean bias (Altman Bland) of +10.9%, -88.9%, -14.9%, Spearman's correlation coefficients of 0.72, 0.88, 0.90 for TgAb, TPOAb and TRAb respectively. Assay concordance for positive/negative results were TgAb (82.0%), TPOAb (92.8%) and TRAb (90.4%), with Cohen's kappa values of 0.64, 0.85 and 0.80 respectively. Non-parametric estimates of upper reference limits (97.5th) of the local volunteer subjects (n=158, M:F ratio=1:2, age range 19-65 yrs old, median age 32 yrs old) were TgAb (107 IU/mL) were TPOAb (30 IU/mL) and TRAb (0.82 IU/L) respectively; distributions were non-Gaussian, with ≥ 98 % of the population falling under manufacturer's upper reference limits of TgAb (115 IU/mL), TPOAb (34 IU/mL) and TRAb (1.75 IU/L). **Conclusion:** Overall, Roche TgAb, TPOAb and TRAb assays showed acceptable analytical performance and represent good alternatives to our current Brahms assays. Notwithstanding wide differences in absolute results between different assay methodologies, substantial concordance was observed with the use of manufacturers' method-appropriate cutoffs. We validated the manufacturer's upper reference limits and found them applicable to our local population. Laboratories looking to switch thyroid autoantibody assays should carefully evaluate the impact of change on patient testing and transferability of the manufacturer's expected values to its own patient population.

## A-199

### Quantitative Proinsulin Assay by Electrochemiluminescence on the Meso Scale Diagnostics Platform

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**Background**: Proinsulin is the precursor of insulin and c-peptide. Proinsulin measurements, in conjunction with insulin and c-peptide, are useful in the diagnostic workup of hypoglycemia and suspected insulinomas. **Objective:** Develop an assay for proinsulin quantitation in human plasma using the electrochemiluminescent multi-array technology from Meso Scale Diagnostics (MSD, Rockville, MD). **Method:** The proinsulin assay is a sequential two-site electrochemiluminescent laboratory-developed test. A biotinylated mouse monoclonal antibody against insulin and proinsulin is

bound to a streptavidin coated 96 well plate, followed by a two hour 25 mcL sample incubation at room temperature. After a wash step, there is a one hour incubation with a SULFO-TAG™ labeled goat polyclonal antibody. After another wash step, an MSD read buffer, containing tripropylamine, reacts with the SULFO-TAG and the electrochemiluminescent signal is captured by the CCD camera of the MESO QuickPlex SQ 120(MSD QuickPlex) instrument. The signal is directly proportional to the amount of proinsulin in the sample. The assay is calibrated against the WHO 1st International Standard for Human Proinsulin (NIBSC code: 09/296). The performance characteristics of the assay were established using at least three different reagent lots. Method validation included determination of imprecision, limits of detection and quantification, analytical measurement range (AMR), accuracy by spike recovery, interferences, effect of antigen excess, and a method comparison with the current Mayo laboratory-developed assay. **Results:** Intra-assay and inter-assay imprecision on human EDTA plasma pools (1.4-27 pmol/L) ranged from 2.0-3.4% and 6.6-10.3%, respectively. The assay limit of detection was 0.03 pmol/L, using calculations suggested by International Union of Pure and Applied Chemistry Compendium of Analytical Nomenclature. A precision profile (~0.1-2.6 pmol/L) established a limit of quantitation of 0.6 pmol/L (%CV=15.3). The AMR was 0.6-350 pmol/L (slope of 0.99, intercept of -0.3, and $R^2$ of 0.98). Spike recovery using WHO 1st International Standard for Human Proinsulin in human EDTA plasma was 93% (range 88-97%). The assay was not affected by concentrations of hemoglobin ≤1228 mg/dL, triglycerides ≤942 mg/dL, or bilirubin ≤57mg/dL. Antigen excess did not affect the assay up to 3234 pmol/L. Competition studies with insulin showed that an insulin concentration up to 3472 pmol/L would not affect proinsulin recovery. Insulin, c-peptide, and Lispro do not cross-react in the assay. Stability studies on freshly collected EDTA plasma showed proinsulin is stable for 8 hours ambient, 3 days refrigerate, and 90 days frozen. Comparison with the in-house proinsulin assay (n=94, range: 1.2-350 pmol/L) showed an $R^2$ of 0.98, slope of 0.99 and intercept of 0.22 by Passing-Bablok regression fit. The reference interval for proinsulin was established by testing 94 healthy individuals (29 males and 65 females) and calculated using quantile regression to be 3.6-22 pmol/L. **Conclusion:** We have developed a proinsulin electrochemiluminescent assay on the MSD QuickPlex standardized against the WHO 1st International Standard for Human Proinsulin. The following advantages are observed when compared to the current in-house assay: broader analytical measurement range (0.6-70 pmol/L to 0.6-350 pmol/L); 10-fold decrease in sample volume (250 mcL to 25 mcL); and shorter turn-around time (1.5 days to 6 hours).

## A-200

### Validation of intact hCG AutoDELFIA assay for use in urine samples

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**Background:** This analysis sought to examine the analytical performance characteristics of an intact human chorionic gonadotrophin (hCG) assay (Perkin Elmer) for use on urine samples on the AutoDELFIA analyser. It was desired that performance would be suitable to enable analysis of hCG concentration in biobanks of urine samples from early pregnancy and to assign concentration to hCG standards used for validation of home pregnancy tests. **Methods:** Limit of blank and Limit of detection were determined following the principles outlined by Pierson-Perry, using 2 reagent lots, 1 instrument system (AutoDELFIA 1235 autoanalyser), 3 test days, a pooled blank sample, 5 low level samples and at least 7 replicate measurements per sample for each reagent-day combination. The pooled blank sample was created by pooling urine from non-pregnant women and passing through an anti-hCG immunoadsorption column to remove all hCG. Samples were prepared from dilution of WHO 5$^{th}$ International Standard for intact hCG. Instrument signals were converted to analyte values through offline calibration to avoid censoring of data for blank samples. Limit of quantitation was calculated via a total error approach, and used 8 low level samples. High dose hook, assay drift, cross-reactivity and interfering substances profile were also examined, as was effect of sample dilution. **Results:** Limit of blank for hCG assay was 0mIU/ml for each reagent lot. Low level sample results (0.25, 0.5, 1, 2, 3mIU/ml, n=28/sample) were used to determine limit of detection as being 0.17mIU/ml. A minimum of 60 repetitions/sample were required to determine limit of quantitation. Total Error was >36% at very low hCG concentrations (0.25, 0.5, 1mIU/ml), whereas total error was <17% for hCG concentrations of 2, 3, 5, 10, 25mIU/ml; so limit of quantitation was deemed to be 2mIU/ml. The linear range of the assay was 0-5000mIU, with samples of higher concentration requiring dilution in order to return accurate results. Dilution of samples retuned results that were 104%±3.13 from expected concentration. No high dose hook or assay drift was observed, nor was there cross-reactivity to species with high homology (LH, FSH, TSH). **Conclusion:** The intact hCG AutoDELFIA assay was found to have suitable analytical performance for use in urine samples and for assigning concentration to reference standards.

## A-201

### Interference in 25OH Vitamin D Assay in a patient with Multiple Myeloma Disease

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**Background: :** Currently automated immunoassays are the most commonly techniques to measure Vitamin D (25-OH VitD). Different studies reported possible interferences in Multiple Myeloma Disease due to immunoglobulins present in the patient' s serum, wich could lead to a wrong laboratory result and also an erroneous diagnosis. The possibility of methodological interference must be taken into account when: the laboratory result shows lack of coherence with the clinical presentation, the presence of an unusual analyte concentration and also discordant results measured by different analytical methods. **Methods:** We reported a case of a 74 years old male with Myeloma disease, he presented high levels of 25-OH VitD concentration without any clinical sign of toxic levels. He denied any oral Vitamin D supplements or any other multivitamin preparation and neither sun exposure. The following analytes were: PTH and 25OH VitD (Reference Values (RV): Sufficiency more than 30 ng/mL, Deficiency minor than 10 ng/mL, insufficiency 10-30 ng/mL, toxicity more than 100.0 ng/mL ) were processed by Architect i2000 (CLIA by Abbott), Gamma Globulins (Nephelometry by Beckman), Calcium and Phosphorus (Colorimetric and *Ammonium phosphomolybdate* respectively by Beckman), Alkaline phosphatase (UV kinetic by Beckman), and Reumathoid Factor (Aglutination).To confirm the presence of interferences the serum was diluted to check linearity and then treated with polyethylene glycol (PEG) (25%), to separate by precipitation high molecular weight forms. **Results:** The patient showed 25-OH VitD concentrations greater than 150.0 ng/mL. Non-linearity dilutions (Dil) (Dil 1:2= 17.8 ng/ml, Dil 1:5= 17.4 ng/mL) were found, wich suggest the presence of interferences. The 25OH VitD concentration post treatment with PEG was 17.6 ng/ml, wich confirm the presence of immune complexes. Those results were confirmed in another sample of the patient (25-OH VitD:180 ng/mL and 25 OH VitD post PEG:16.4 ng/ml). On the other hand the result of IgG: 6520 mg/dL (RV: 800-1700) and the protein electrophoresis showed a monoclonal peak in Gamma 4.76 g/dL, confirm the high concentration of immunoglobulins. **Conclusion:** Assay interference should be considered in unexpected abnormal results of 25OH Vit D levels in presence of Myeloma disease. Laboratory´s staff should contemplate the use of additional tools to detect and eliminate these kind of artifacts. Communication with physicians is very important for patient follow-up.

## A-202

### An evaluation of hemoglobin A1C measurement by dried blood spots

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**Background:** Diabetes is a chronic metabolic disorder of sustained high blood sugar levels that carries a high cost in resources, quality of life and mortality. Due to changing lifestyles and diets, its global prevalence is growing especially in low- and middle-income countries. Glycated hemoglobin ($HbA_{1C}$) levels represent a patient's mean serum glucose level over the previous 3-4 months and is the preferred test for diabetes diagnosis and monitoring. Clinical testing by dried blood spot (DBS) is an approach that has numerous advantages over conventional phlebotomy including a small volume requirement, simple collection that can be performed by the patient at home, nonhazardous transportation and handling, compact storage, and long stability in the absence of refrigeration. The measurement of $HbA_{1C}$ from DBS is therefore attractive for widespread diabetes testing and monitoring, especially in locales with access challenges to testing facilities. Here, we assessed DBS as the specimen of choice for $HbA_{1C}$ measurement by employing a simple extraction method in combination with the Roche Cobas c 513 analyzer (Roche Diagnostics). **Methods:** 65 µL EDTA whole blood samples rewarmed to room temperature were spotted on Whatman® 903 Protein Saver cards (Sigma-Aldrich) and air dried for 4-12 hours. Samples were then extracted by 6.00 mm diameter hole punches and resuspension in 1 mL of Hemolyzing Reagent for Tina-quant® $HbA_{1C}$ Gen. 3 immunoassay (Roche Diagnostics) which contains tetradecyltrimethylammonium bromide detergent. After 30 min at room temperature with periodic gentle inversions, samples were spun for 1 min at 14,000 rpm in a microcentrifuge for final sample extraction. The recovered supernatants were aliquoted to false bottom tubes for testing on the Roche Cobas c 513 analyzer via the hemolysate application. Within run precision of this DBS method was evaluated using 20 repetitions of a normal sample (5.8%$HbA_{1C}$) and a pathological sample (10.5%$HbA_{1C}$). Method accuracy was determined by spotting and extracting

stored proficiency samples from the College of American Pathologists. For direct comparison with routine whole blood testing, 40 samples representing a range of 4.6% to 14.0%$HbA_{1C}$ as measured by the former method were spotted, extracted and analyzed. **Results:** Using the DBS extraction procedure, within run precision of $HbA_{1C}$ measurement was 0.4% and 0.8%CV for normal and pathological leftover patient samples, respectively. Measurement of proficiency survey samples by DBS extraction found a low average bias of 0.01% ($y = 1.03x - 0.20$; $R^2 = 0.999$). Strong correlation was also observed in comparison runs between the DBS method and routine phlebotomy-based whole blood $HbA_{1C}$ testing using the same instrument ($y = 0.98x + 0.21$; $R^2 = 0.999$). In these runs, samples showing relatively high bias were scattered across the measuring range and showed no clear systematic pattern. Furthermore, the associated biases remained within the total allowable analytical error (2.0%) with absolute biases no greater than 0.4%$HbA_{1C}$. **Conclusion:** The simple DBS extraction method partnered with the Roche Cobas c 513 is reproducible, accurate and robust for $HbA_{1C}$ analysis. This supports the implementation of this convenient specimen collection approach for wide-reaching diabetes testing at a population level.

## A-203

### Development of prototype renin concentration assay which well-correlate with renin activity under the treatment with direct renin inhibitor

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Background: Renin controls blood pressure via renin-angiotensin-aldosterone system. Measuring renin concentration is useful to screen primary aldosteronism from large number of hypertensive patients. There are two forms of assays to measure renin in plasma currently used in clinical settings, plasma renin activity (PRA) and plasma renin concentration (PRC). Although PRA assays offer better sensitivity, they also have disadvantages such as the dependence on the plasma concentration of renin substrate angiotensinogen and the difficulties in sample management. On the other hand, PRC assays have advantages in terms of rapidity and easy sample management and several active renin specific PRC assays have been commercially available. However, these PRC assays are known to have poor correlation with PRA under treatment with direct renin inhibitor, aliskiren, because renin specific antibody used in the current commercial PRC assays detects aliskiren bound renin and prorenin as active renin, despite their inactivation by aliskiren binding. In this study, we aimed to establish PRC assay which highly correlates with PRA even for the patients under the treatment with direct renin inhibitor. Methods: Anti-renin antibodies were established by mice immunization and renin specific antibodies were selected based on specificity to renin. These antibodies were characterized for the reactivity to renin in the presence or absence of aliskiren. Renin specific sandwich ELISA with these antibodies was evaluated by reactivity to renin and prorenin and correlation with renin activity. Result: Several monoclonal antibodies which showed high specificity to renin were successfully obtained. Cross reactivity to recombinant prorenin of these antibodies was less than 10% of reactivity to recombinant renin. It was found that one of the highly renin-specific antibodies lost reactivity to recombinant renin in the presence of aliskiren in the concentration dependent manner, while the reactivity of other antibodies was not affected. Moderately renin-specific antibody which showed approximately 50% of cross reactivity to prorenin was also obtained. The sandwich ELISA established using highly renin specific and aliskiren-sensitive antibody and moderately renin specific antibody showed not only high specificity to active renin, but also aliskiren sensitivity. Further analysis revealed that this aliskiren sensitive ELISA assay showed high correlation with renin activity as measured by in-house renin activity assay using fluorescent renin substrate even with aliskiren. Conclusion: We have developed an unique renin specific monoclonal antibody that loses reactivity in the presence of direct renin inhibitor, aliskiren. The measurement of recombinant renin by the sandwich ELISA developed with our aliskiren sensitive antibody showed striking correlation with those of in-house renin activity assay. Although clinical significance has yet to be revealed, our aliskiren sensitive, highly renin-specific antibody offers promising tool to develop PRC assay which reflects true plasma renin activity, and may be useful not only for the diagnosis of primary aldosteronism, but also for monitoring therapeutic effects of direct renin inhibitors.

## A-204

### Frequency of insulin resistance assessed by Quantose-IR©, HOMA index and triglycerides/HDL-c ratio.

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**Background**. Overweight, obesity and associated co-morbidities diabetes and cardiovascular disorders have increased dramatically in Mexico. Insulin resistance (IR) is an important factor for development of diabetes. Quantose-IR© is a non-glycemic test to identify IR. **Objective.** To assess the frequency of IR by Quantose-IR©, HOMA index, and triglycerides/HDL-c (T/H) ratio, in ambulatory patients from two laboratories in Mexico City. **Methods**. A laboratory information system database search was performed to identify patients that requested a chemistry profile and Quantose-IR© (Metabolon Inc.) from January 2016 to March 2017. Patients age 18 and older were included; those with a serum glucose ≥126 mg/dL were excluded. Quantose-IR was performed at a Mexican reference laboratory and provides an IR index based on plasma concentration of α-hidroxibutiryc acid, oleic acid, 1-linoleoylglycerophosphocholine measured by UHPLC-MS-MS, plasma insulin measured by chemiluniscence and a Metabolon proprietary algorithm. Serum glucose and lipids were analyzed locally in AU5800 and Lx20 instruments (Beckman-Coulter). Cut-off values for IR were >63 for Quantose-IR, >2.5 for HOMA index and for T/H was >2.5 for women and >3.5 for men. Statistical differences were evaluated by chi2 test for categorical variables and Mann-Whitney test for quantitative variables. **Results.** Patients included were 708, 360 (51%) were females. Median age was 46 years, ranging from 18 to 85 years. The overall IR frequency was 78% for Quantose-IR, 52% for TG/HDLc ratio and 45% for HOMA index, with p-values of <0.001 and <0.0001 for Quantose vs. T/H ratio and HOMA index respectively. The frequency of IR when patients were classified according to glucose and insulin concentrations is summarized in the following table.

| | N | Quantose | HOMA index | T/H ratio |
|---|---|---|---|---|
| Normal glucose and normal insulin | 481 | 72% | 21 | 54 |
| Abnormal glucose and normal insulin | 147 | 88% | 72% | 61% |
| Normal glucose and abnormal insulin | 45 | 100% | 100% | 75% |
| Abnormal glucose and abnormal insulin | 35 | 100% | 100% | 80% |

**Conclusion.** Quantose-IR identifies more patients as IR notably in normal glucose and insulin patients, HOMA index is best used when insulin concentration is abnormal. T/H ratio had the lowest performance for identifying IR.

## A-205

### Comparison of Five TSH-Receptor Antibody Assays in Graves' disease. Results from an observational study

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**Background:** Early diagnosis and relapse prediction in Graves' disease [GD] may influence treatment. We assessed the abilities of four TSH-receptor antibody tests [TRAb] and one cAMP bioassay to predict relapse of GD. **Methods:** Observational study investigating patients presenting with GD at a Swiss hospital endocrine referral center or an associated endocrine outpatient clinic. Main outcomes were diagnosis and relapse of GD after stop of anti-thyroid drugs. We used Cox regression to study associations of TRAb levels with relapse risk and calculated area under the receiver operating characteristics curve [AUC] to assess discrimination. Blood draws took place as close as possible to treatment initiation. **Results:** ROC curve analysis revealed AUCs ranging from 0.90 (TSAb Biossay) to 0.97 (IMMULITE TSI) for the diagnosis of GD. Highest sensitivity (94.0%) was observed for IMMULITE and RSR TRAb Fast while the greatest specificity (97.9%) was found with the EliA anti-TSH-R. GD relapse was studied using Cox regression analysis comparing the highest versus the lower quartiles. The highest hazard ratio [HR] was found for BRAHMS TRAK (2.98, 95% CI 1.13 - 7.84), IMMULITE TSI (2.40, 95% CI 0.91 - 6.35), EliA anti-TSH-R (2.05, 95% CI 0.82 - 5.10), RSR Fast TRAb (1.80,

95% CI 0.73 - 4.43), followed by RSR STIMULATION (1.18, 95% CI 0.46 - 2.99). Discrimination analyses showed respective AUCs of 0.68, 0.65, 0.64, 0.64, and 0.59. **Conclusion:** The assays tested had good diagnostic power and relapse risk prediction with few differences among the new assays.

## A-206

### Do we need to chill samples for renin activity?

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**Background**:
Collecting blood on ice for renin determination reportedly results in spuriously high results due to activation of prorenin into renin which converts angiotensinogen into angiotensin 1. However when determining plasma renin activity, samples are requested to collect into pre-chilled EDTA tubes. Our objective was to assess whether samples need to be taken into pre-chilled tubes in actual condition.
**Methods:**
A prospective cross sectional study was performed. Patients who came for plasma renin and aldosterone assay were taken as the study sample. Peripheral venous blood was collected from 22 patients in the seated position after 2 hrs of ambulation between 08:00 and 10:00 h. From each patient blood was collected into 2 tubes containing K-EDTA (pre-chilled and room temperature EDTA tubes). Blood taken into pre-chilled EDTA tube was immediately centrifuged at 4 $^0$C and the other tube was processed at room temperature. The interval from collection to commencement of incubation averaged between 1 to 2 hours. Radioimmunoassay of angiotensin 1 was used to determine plasma rennin activity. Angiotensin 1 was measured after 1 hr incubation at both 4$^0$C and 37$^0$C. The assay was performed in duplicates. The plasma rennin activity was calculated by the difference in angiotensin 1 found in 2 samples. For the statistical analysis, values were reported as mean ± SD (Paired t test). The difference at $P < 0.05$ was considered significant.
**Results:**
There was no significant difference in the mean value for plasma renin activity in samples whether processed at room temperature (3.29 ± 2.77) or 4$^0$C (3.05 ± 2.75). Samples handled at room temperature had a higher basal angiotensin 1 value (2.03 ± 4.16 Vs 1.51 ± 3.26) but, the difference was not significant.
**Conclusion:**
Our data indicate that use of pre-chilled EDTA tubes and refrigerate centrifuge is unnecessary to determine plasma rennin activity and at 4$^0$C, cryoactivation of prorenin does not occur rapidly.
**Keywords**
Renin activity, chilled EDTA tubes, prorenin

## A-207

### Requirement for age-specific peak cortisol references to insulin-induced hypoglycaemia in children.

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**Background:** The insulin tolerance test (ITT) is frequently used for simultaneous evaluation of the hypothalamic-pituitary-adrenal axis and growth hormone secretion in children. In contrast to extensive published literature pertaining to GH response following ITT, only few reports have considered the magnitude of cortisol response to this test in pediatric population. Reference range is not clearly established for peak cortisol response to ITT despite limited data suggesting an effect of age on peak cortisol. **Objective:** To determine whether peak cortisol response to insulin hypoglycemia test in children is related to age and to try to establish pediatric reference data. **Design:** The present study was a retrospective cohort study. **Methods:** We performed a retrospective analysis of children and adolescents submitted to insulin tolerance test in a laboratory referral center over a 5-year period (2012 - 2017). Inclusion criteria were age ≤ 18 years, adequate hypoglycemia, defined as a glucose nadir ≤ 2.2 nmol/L (≤ 40 mg/dL) (4, 7) and a normal response of cortisol to the test. A normal response to the test was defined as a peak cortisol (maximum absolute concentration) at any time of the test ≥ 400 nmol/L (14.4 µg/dL). Patients with known or suspected organic hypothalamic-pituitary diseases and patients receiving glucocorticoid medication were excluded. One hundred and twenty-four subjects (86 males) met the criteria. Blood samples were collected at time 0, 30, 60, 90 and 120 min in relation to insulin bolus injection (0.075 - 0,15 U/kg). Glucose, cortisol and growth hormone were measured in all samples. **Results:** One hundred and twenty-four patients were eligible for inclusion in our study, 69% of which were male. Peak cortisol was inversely correlated with age (r= -03297, p= 0.0002). The median and 5$^{th}$ centile peak cortisol value were, respectively, 629 nmol/L (22.8 µg/dL) and 500 nmol/L (18 µg/dL) in children < 12 years as compared with, respectively, 564 nmol/L (20.4 µg/dL) and 457 nmol/L (16.5 µg/dL) in children ≥ 12 years. Median cortisol peak was significantly higher in younger patients compared to older patients (P=0.0004). **Conclusion:** The peak cortisol is age related. A single peak cortisol threshold in children of all ages is not appropriate and will result in overdiagnosis of adrenal insufficiency in adolescents.

## A-208

### In Pursuit of an Optimal Vitamin D Assay in the Era of High Patient Volume and Complexity

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**Background:** The Institute of Medicine recommends total 25-hydroxyvitamin D (25-OHD) testing to screen high risk patients for deficiency and monitor supplementation therapy. Numerous factors (i.e. test volume, laboratory type and equipment, patient population) should be considered when determining the best methodology for 25-OHD testing. LC-MS/MS is the gold standard for measuring 25-OHD in the clinical laboratory, allowing for quantification of 25-OH D2/D3and respective epimers, however, the FDA only recommends reporting total 25-OHD. In practice, LC-MS/MS is manual, highly complex and time consuming. There are numerous 25-OHD immunoassays (IA), but historically these assays were subject to numerous interferences from a number of sources including 25-OHD metabolites and lipids. Herein, we describe a multicenter study comparing NIST standardized LC-MS/MS 25-OHD assays with three contemporary immunoassays in a complex patient population. **Objective:** To perform a multicenter study comparing two LC-MS/MS 25-OHD methods with three contemporary immunoassays in complex patient populations. **Methods:** 25-OHD in patient samples was quantified by three contemporary IA methods (Abbott Architect 25-OH Vitamin (New formulation), Roche Elecsys Vitamin D total II, and BioRad BioPlex 25-OH Vitamin D). Results were compared to one of two NIST standardized LC-MS/MS methods (which detect 25-OH-D2 (D2) and 25-OHD3 (D3)) at two clinical laboratories (University of Kentucky (UK) and/or Seattle Children's Hospital (SCH)). The 3-epi 25(OH)D (epimer) was chromatographically resolved by SCH-LC-MS/MS. Clinical information was obtained from patients' Electronic Health Record. Statistical analyses were performed in EP Evaluator. **Results:** The table shows a comparison of 25-OHD results between contemporary IA and LC-MS/MS methods in complex population and subpopulations. **Conclusion:** The 25-OHD results correlated well between LC-MS/MS and contemporary immunoassay methods in the total population; however, some subpopulations had poor correlation. Laboratories should consider the contribution of 25-OHD isomers, epimers and other minor metabolites when choosing a Vitamin D assay in complex patient populations.

## A-209

### Molecular heterogeneity of Macroprolactin in samples suspected of false hyperprolactinemia.

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**Background:**
Circulating prolactin (PRL) exists in several molecular forms with different biological activities, in some instances making PRL assay and its diagnostic value unreliable. Macroprolactin (macro-PRL), a 150 kDa molecular weight (MW) form, is known to have low biological activity which may lead to misdiagnosis and inappropriate treatment in patients with suspected hyperprolactinemia. Polyethylene glycol precipitation (PEGP) is widely used to identify the presence of macro-PRL, but up to 20% of monomeric PRL is lost during this procedure and PEG itself can interfere with some PRL immunoassays. Additionally, PEGP has been reported to give false positive results for macro-PRL in patients with increased serum globulins (IgG myeloma and HIV patients). The aim of this study is to identify different PRL variants in samples suspected of macro-PRL using gel filtration chromatography (GFC).
**Methods:**
Twelve samples obtained from Nichols Institute (San Juan Capistrano, CA) with measured PRL concentrations prior to and following PEGP were analyzed using GFC analysis. Briefly, 100 uL of sample or protein markers (molecular weights ranging from 12.4 to 200 kDa) were applied to a Superdex 200 column (Pharma-

cia, Sweden). Forty fractions of 0.5 mL each were collected per patient sample at a flow rate of 0.4 mL/min using PBS containing 1% (w/w) bovine serum albumin as the mobile phase. Elution of marker proteins was detected by recording absorbance at 280 nm. Prolactin concentrations of all GFC fractions were measured using ELISA (R&D systems, Minneapolis) according to the manufacture's protocol.
**Results:**
A total of 12 samples were subjected to GFC analysis. Nine samples had high levels of total PRL (30.2 - 1469 ng/mL) whereas 3 samples had total PRL levels within the reference intervals (21.4 - 29.4 ng/mL). All samples positive for macro-PRL by PEGP analysis (n=5) exhibited high-molecular-weight PRL (>150kDa) and mid-molecular-weight PRL (30-150kDa), with one of the samples also exhibiting low-molecular-weight PRL (<30kDa). In macro-PRL negative samples by PEGP (n=5), all showed mid-molecular-weight PRL, one sample had high-molecular-weight PRL, and one sample exhibited low-molecular-weight PRL as well. Two samples within the PEGP indeterminate zone (60-40% recovery) had mid-molecular-weight PRL, one sample exhibited low-molecular-weight PRL, whereas the other sample exhibited high-molecular-weight in addition. PEGP failed to identify a small amount of high-molecular-weight PRL in one sample seen by GFC analysis.
**Conclusion:**
For the majority of our samples (11 out of 12), PEGP results agreed with GFC results in identifying macro-PRL. GFC analysis showed marked molecular heterogeneity for macro-PRL as defined by PEGP analysis. Mid-molecular weight-PRLs were present in every sample and both high-molecular weight-PRL and mid-MW-PRL had diverse patterns, but their clinical significance and physiological roles remained unclear. This is the first study to report molecular heterogeneity for macro-PRL.

## A-210

### Can baseline cortisol predict short synacthen test response?

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**Background**
The short synacthen test (SST) is the dynamic function test most widely used to assess hypothalamic pituitary adrenal axis. It is possible that a single basal cortisol value can predict the response of this dynamic test. Our aim was to determine a morning baseline cortisol value that could predict SST response.
**Methods**
We conducted a retrospective analysis of short synacthen test results (using Advia Centaur XP/ Siemens) of samples received to the radioimmunoassay laboratory in the National Hospital of Sri Lanka from May 2017 to October 2017. Patients who were acutely ill or in intensive care and on glucocorticoid therapy were excluded and 98 patients remained for analysis. The SST was considered to have an inadequate response when 30 min cortisol level was below 550 nmol/L. ROC curve was generated to determine a predictive value of basal morning cortisol for failing SST.
**Results**
Seventy five patients had adrenal insufficiency and 23 patients were adrenal sufficient. ROC curve had a good overall predictive value (AUC - 0.814; 95% confidence Interval 0.715 - 0.914). Baseline cortisol level predicting failing the SST with 100 percent specificity was 132 nmol/L (sensitivity of 36%). All the patients with a basal cortisol level of 442 nmol/L or above had passed the test (Sensitivity - 100% and specificity - 27%). A basal cortisol value of 256 nmol/L was recognized to predict adrenal insufficiency with sensitivity of 90% and specificity of 67%.
**Conclusion**
A single value with a high specificity and sensitivity which can predict the outcome of SST cannot be defined. If morning basal cortisol level is either < 132 nmol/L or > 442 nmol/L, it is not necessary to perform SST and it would have prevented 58 (58.6%) SST. Therefore basal morning cortisol might help in avoiding unnecessary SST and provides a cost-effective approach.
**Keywords**
Baseline cortisol, short synacthen test, adrenal insufficiency
Background

## A-211

### Free 25 Hydroxy Vitamin D by LC-MS/MS: Reference Intervals in Healthy Adults and Observations in Pre-/Post-Menopausal Women

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**Background:** Serum 25-hydroxy vitamin D (25OHD) is widely used as a marker of vitamin D sufficiency. In circulation, 25OHD is tightly bound to proteins with less than 0.1% circulating in free form. It has been demonstrated that the majority of human cells respond to the free, rather than protein-bound, form of 25OHD. Therefore, measurement of free 25OHD (F25OHD) may be a relevant biomarker for assessing the physiologically active fraction of vitamin D.
**Methods:** We developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for F25OHD, evaluated its performance and established reference intervals for healthy adults. Sample preparation for F25OHD was performed as follows: F25OHD (F25OHD2 and F25OHD3) were separated from the protein bound fraction using size exclusion based technique; stable isotope labeled internal standard was added to the samples, 25OHD was derivatized and analyzed by LC-MS/MS. Two mass transitions were monitored for F25OHD2, F25OHD3, and the internal standards; ratio of the mass transitions was used to confirm specificity. Concentrations of calcium (Ca) and parathyroid hormone (PTH) were determined using Cobas 8000 modular analyzer (Roche Diagnostics, Indianapolis); total 25OHD (T25OHD2 and T25OHD3) was measured using LC-MS/MS. The method was compared to a commercial F25OHD ELISA (Future Diagnostics, The Netherlands). Reference intervals were established using samples from 120 self-reported healthy adult volunteers (52 men, 68 women; age range 20-63 years; 111 Caucasian, 6 Hispanic, 3 Asian; 63% collected during summer/fall months, 37% collected during winter months). Mean age was 38.5 and 34.5 for men and women, respectively; number of samples by age group was: 41, 34, 27, and 18 (20-30, 31-40, 41-50, 51-63 years old, respectively); 55 samples were from premenopausal women (PW) and 13 samples were from postmenopausal/perimenopausal women (PPW). Concentrations of Ca, PTH, and (T25OHD2+T25OHD3) in the samples were within the corresponding reference intervals.
**Results:** For F25OHD2 and F25OHD3, the lower limit of quantitation was 0.005 ng/mL and total imprecision at concentrations above 0.01 ng/mL F25OHD was <15%. Nonparametric reference intervals for F25OHD and percent F25OHD were 0.024-0.080 ng/mL and 0.08-0.18%, respectively. Statistically significantly higher concentrations of T25OHD (p=0.0089) and F25OHD (p=0.049) were observed in samples collected during summer/fall than during winter. No statistically significant difference in F25OHD concentrations were observed between men and women or among the age groups. While PPW had higher concentrations of T25OHD than PW (p=0.07), no statistically significant difference was observed in the distribution of concentrations of F25OHD between PPW and PW (p=0.543). However, statistically significantly lower percent F25OHD was observed in PPW than in PW (p=0.033).
**Conclusion:** In summary, we developed a LC-MS/MS method for measurement of F25OHD and established reference intervals for F25OHD and percent F25OHD in healthy adults. Lower percent F25OHD observed in PPW is likely explained by higher concentrations of binding proteins in this population, and could be a contributing factor to the higher incidence of osteoporosis observed in postmenopausal women.

## A-212

### Evaluation of the cobas c 513 analyzer for HbA1c assay

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**Background:** Hemoglobin $A_{1c}$ ($HbA_{1c}$) is an essential biomarker for monitoring glycemic control in diabetic patients and is also used in the diagnosis of diabetes mellitus. Therefore, reliable and efficient methods are required for $HbA_{1c}$ quantification. This study evaluated the analytical performance of the Tina-quant® $HbA_{1c}$ third generation immunoassay on the **cobas c** 513 analyzer.
**Methods:** Precision was assessed according to Clinical and Laboratory Standards Institute EP05 guidelines, using quality controls (n=2) and patient samples (n=4), which were analyzed in duplicate twice a day for 21 days, on the **cobas c** 513 (throughput: 400 samples/hour). Method comparison was performed against two routinely used high-performance liquid chromatography (HPLC) analyzers (D-100 and Variant II, BioRad laboratories). Accuracy was evaluated against 8 external quality assurance samples (European Reference Laboratory for Glycohemoglobin) with IFCC-assigned

target values (31.4-99.2 mmol/mol). Analytical interference by bilirubin, triglycerides and common Hb variants (Hb AC, AD, AE, AS) on $HbA_{1c}$ quantification was assessed. Data are reported for $HbA_{1c}$ values in IFCC units (mmol/mol) unless stated. **Results:** The $HbA_{1c}$ assay demonstrated good precision, with coefficients of variation (CV) lower than 1.13% and 1.73% for $HbA_{1c}$ values expressed in NGSP (%) and IFCC units, respectively (**Table**). Good correlation of the $HbA_{1c}$ assay was observed with both HPLC systems (D-100: y=0.951x +2.757, r=0.997, n=100; Variant II: y=0.997x + 1.904, r=0.998, n=100). The analysis of samples with IFCC-assigned values showed a good accuracy of the method; relative biases ranged from -0.2% to 3.4%. No interference by bilirubin (0-352 µmol/L, relative bias -1.4% to 1.6%), triglycerides (0-20.6 mmol/L, relative bias 4.0% to 0.8%) and common Hb variants was observed. **Conclusion:** The $HbA_{1c}$ assay on the **cobas c** 513 analyzer demonstrated a good analytical performance, and is therefore suitable for routine use in clinical chemistry laboratories.

| Precision of the HbA1c assay on the cobas c 513 analyzer | | | | | |
|---|---|---|---|---|---|
| Sample | Mean value | Repeatability (CV, %) | Between-run precision (CV, %) | Between-day precision (CV, %) | Intermediate precision (CV, %) |
| HbA1c values in % | | | | | |
| Sample 1 | 5.80 | 0.46 | 0.62 | 0.75 | 1.08 |
| Sample 2 | 6.07 | 0.67 | 0.61 | 0.65 | 1.12 |
| Sample 3 | 7.90 | 0.49 | 0.79 | 0.63 | 1.12 |
| Sample 4 | 11.61 | 0.62 | 0.63 | 0.71 | 1.13 |
| QC sample (low-level) | 5.74 | 0.57 | 0.46 | 0.41 | 0.84 |
| QC sample (high-level) | 11.13 | 0.54 | 0.21 | 0.91 | 1.08 |
| HbA1c values in mmol/mol | | | | | |
| Sample 1 | 39.9 | 0.74 | 0.98 | 1.21 | 1.73 |
| Sample 2 | 42.9 | 1.03 | 0.95 | 1.01 | 1.73 |
| Sample 3 | 62.9 | 0.69 | 1.09 | 0.87 | 1.55 |
| Sample 4 | 103.5 | 0.77 | 0.78 | 0.87 | 1.40 |
| QC sample (low-level) | 39.2 | 0.90 | 0.71 | 0.68 | 1.34 |
| QC sample (high-level) | 98.2 | 0.68 | 0.25 | 1.14 | 1.35 |

## A-213

### Three alternative makers of hyperglycemia for early detection of diabetes: glycated albumin, 1,5-anhydroglucitol, and fructosamine

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**Background:** Glycated albumin (GA), 1,5-anhydroglucitol (1,5-AG), and fructosamine have recently attracted considerable interest as markers of hyperglycemia. However, these alternative markers are not understood well enough yet for use in the clinic. In this study, we assessed their potential utility in the early identification of hyperglycemia. **Methods:** We conducted an analysis of 5,800 participants who underwent healthcare study between August 2013 and September 2014 and had no history of diagnosed diabetes mellitus (DM). All of the tests were performed at the laboratory department in the Kangbuk Samsung Hospital Total Healthcare Center in Seoul, Korea. Blood specimens were sampled from the antecubital vein after an 8-hour fast. Serum GA, 1,5-AG and fructosamine levels were measured by automatic chemistry analyzer (Modular P800; Roche Diagnostics, Tokyo, Japan) using a Lucica GA-L reagent (Asahi Kasei Pharma Co., Tokyo, Japan) based on the enzymatic method; Determiner L 1,5-AG reagent (Kyowa Medex, Tokyo, Japan) based on the colorimetric method; and Fructosamine reagent (Roche Diagnostics GmbH, Mannheim, Germany) based on a colorimetric method, respectively. We divided the study population into normal, pre-DM, and DM groups according to fasting blood glucose (FBG) and HbA1c levels. Among them, 77.0% of the participants had follow-up examinations before July 2017 and 100 participants were newly categorized as DM group. The area under the receiver operator characteristic (AUC-ROC) curves was calculated to determine the ability of three alternative markers to predict hyperglycemia. We then conducted multivariate analysis to estimate DM progression. **Results:** Participants in the DM and pre-DM groups were more likely to be older than those in the normal group, with mean ages of 49.4 ± 9.9 (range, 33-76) and 44.2 ± 9.3 (15-77) vs. 39.7 ± 7.8 (18-77) years, respectively. Mean levels of GA, 1,5-AG, and fructosamine were significantly different among the three groups. At the ROC analysis, estimated cut-off values of GA, 1,5-AG, and fructosamine for the DM group criteria with HbA1c≥6.5% and/or FBS≥126 mg/dL were 13.1%, 11.8 ug/mL and 253 umol/L with good AUC values; 0.849, 0.862, and 0.818. For the pre-DM and DM groups criteria with HbA1c≥5.7% and/or FBS≥100 mg/dL estimated cut-off values were 12.2%, 17.2 ug/mL and 243 umol/L but the AUCs were poor as 0.552, 0.605, and 0.609. On follow-up, 4.8%, 5.7%, and 5.8% of the highest risk quintile groups according to baseline levels of GA, fructosamine, and 1,5-AG had progressed from non-DM to DM group, while the lowest risk quintile of each group exhibited 1.4%, 0.6%, and 1.7% progression. The highest risk group of 1,5-AG showed a higher odds ratio(OR) for the DM progression than those of GA and fructosamine (estimated OR after adjustment for confounding variables: 4.720, 12.509 and 3.667 at the highest risk group of GA, 1,5-AG, and fructosamine). **Conclusion:** The highest risk quintile groups of these three markers were associated with progression to DM. Our results suggest that these markers may be useful alternatives and supporting to the traditional markers in early screening for hyperglycemia.

## A-214

### Multicenter performance evaluation of the Elecsys® Insulin-like Growth FactorI immunoassay and establishment of reference ranges in a large cohort of healthy subjects

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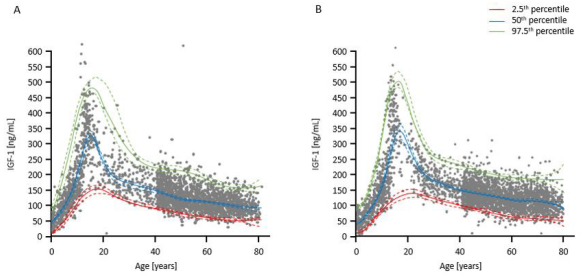
**Background:** Insulin-like growth factor-I (IGF-I) is a biomarker used to assess disorders of the growth hormone/IGF axis. We evaluated the analytical performance of the Elecsys® IGF-I electrochemiluminescence immunoassay and established reference ranges in apparently healthy subjects. **Methods:** Three laboratories evaluated the Elecsys IGF-I assay (Roche Diagnostics) under routine conditions. Experiments were performed on **cobas e** 411 and 601 analyzers. Repeatability (within-run) and intermediate precision (within-laboratory) were assessed according to Clinical Laboratory Standards Institute (CLSI) EP5-A3 guidelines (5-day model, 3 reagent lots [2 per site]). Samples comprised 7 human serum pools and 2 control samples (PreciControl Growth, Roche). Functional sensitivity was evaluated, and method comparisons of Elecsys IGF-I assay (measuring range: 7-1600ng/mL) versus 3 commercial assays were performed (CLSI EP9-A3 guidelines). Clinical evaluations used samples from the LIFE (NCT02550236) and EU Sample Collection studies. **Results:** Samples with moderate/high (55.2-1487ng/mL) and low IGF-I levels (22.2-25.9ng/mL) met predefined acceptance criteria for repeatability, intermediate precision and inter-module precision; CVs for moderate/high samples were 1.0-3.2%, 1.0-6.3% and 3.5-6.7%, after excluding outliers. Functional sensitivities for each lab (CV threshold 20%) were 4.75, 7.23 and 11.30ng/mL IGF-I, respectively. The Elecsys IGF-I assay showed good agreement with IGF-I results from IDS iSYS (Passing-Bablok regression slope, 1.13; intercept, -14.0; Pearson's r, 0.995; n=146), Siemens Immulite 2000 (0.873; -28.8; 0.956; n=135) and Diasorin Liaison (0.859; -14.0; 0.993; n=145). Evaluation of samples from 6698 apparently healthy subjects (age: 3 months-80 years) showed IGF-I concentrations for both sexes increased rapidly from birth, reaching a peak median concentration at 15 years in females (n=3046; 331ng/mL) and 17 years in males (n=3652; 340ng/mL); levels decreased sharply during early adulthood and remained relatively constant in senescence (**Figure**). **Conclusion:** We demonstrate robust analytical performance of the Elecsys IGF-I assay under routine conditions and provide gender-dependent reference ranges based on results from apparently healthy subjects.

**Figure.** Quantile regression of IGF-I serum concentrations in apparently healthy **(A)** females (n=3046) and **(B)** males (n=3652). Plotted curves represent 2.5th, 50th and 97.5th percentiles (solid lines) and associated 95% confidence intervals (dashed lines).




## A-215

### Fractional Excretion of Vitamin D Binding Protein as a Novel Marker of Incipient Diabetic Nephropathy and Vitamin D Status in Subjects with Type 2 Diabetes Mellitus

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**Background:** Vitamin D deficiency (VDD) has been shown to play significant roles in the pathogenesis and complications of Type 2 Diabetes mellitus (T2DM). Vitamin D-binding protein (VDBP), a 58-kDa glycoprotein, is a significant determinant of biologically active levels of 25(OH) Vitamin D (25(OH)D). Studies have shown increased urine excretion of VDBP in patients with diabetic nephropathy (DN) but the exact mechanism of increased VDBP excretion is not clearly understood. The endocytotic receptor pathway in renal tubules is involved in the reabsorption of 25(OH)D and VDBP filtered in the glomerulus and, with the onset of nephropathy in diabetes, we hypothesized that increased urine protein load could affect clearance of VDBP. In this study, we evaluate the utility of the Fractional Excretion of VDBP (FEVDBP) as a novel index of VDD and DN. **Methods:** Levels of 25(OH)D, HbA1c, serum and urine concentrations of VDBP, creatinine were measured in 405 (129M, 276F) T2DM patients. Ratio of urine microalbumin to creatinine was determined to categorize subjects as normoalbuminuric (NAO, ratio <30mg/g); microalbuminuric (MIA, ratio 30-300mg/g) and macroalbiminuric (MAA, ratio >300 mg/g). FEVDBP was calculated as 100 x (UrineVDBP x SerumCreat) / (SerumVDBP x UrineCreat). Univariate and multivariate analyses were used to compare study subjects grouped by Vitamin D status, glycemic control and degree of microalbuminuria**.** **Results:** VDD (<50nmol/L; n = 237) or insufficiency (VI) (50-75 nmol/L; n=84) was prevalent. Urine VDBP concentration increased stepwise with increasing degrees of microalbuminuria. Mean FEVDBP in subjects with normal 25(OH) D, VI and VDD were 5.5, 5.4 and 8.5 respectively; mean FEVDBP in NAO, MIA and MAA were 3.7, 23.3 and 55.9 respectively. Significant correlations of FEVDBP were with age (r=0.38), glucose (r=0.42), HbA1C (r=0.46), urine microalbumin:creatinine ratio (r=0.56) and significant negative correlation with serum albumin (r = - 0.30). Receiver operating Curve (ROC) analyses of the use of FEVDBP for detection of VDD, microalbuminuria and poor glycemic control showed that the areas under the ROC are 0.545, 0.822 and 0.732 respectively. **Conclusion:** Unlike other studies where only urinary concentrations of VDBP was evaluated, we assessed the ratio of VDBP excreted in the urine taking into account its levels in the plasma thereby providing a more accurate measure of filtered VDBP. Increased FEVDBP in MIA and MAA confirms our hypothesis that onset of nephropathy in diabetes increases the clearance of VDBP. We conclude that increased FEVDBP contributes to the mechanisms of VDD in T2DM. The significant associations of FEVDBP with glycemic control and DN suggests that this index could play a wider role in the pathogenesis and/or detection of diabetic complications.

## A-216

### TSH immunoassays: Commutability of EQAS samples and relation to inter-method differences

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**Background:** Selectivity and reference preparations are the basics of a traceability chain for measurands with no reference measurement system available. All immunoassays claim to measure hTSH and use the same WHO IRP, they should produce comparable results. In a method comparison study of the IFCC Working Group for Standardization of Thyroid Function Tests (WG-STFT), 13 out 16 hTSH immunoassays had their standardization set-point within 10% from the all-procedure trimmed mean, however, the average dispersion across all assays was of the order of ~32%. Same results can be observed from EQAS results. Commutability of samples can be also an issue in intermethod differences. The aim of this study was to investigate different start-up materials for EQA samples and the relationship with differences observed in TSH results from EQAS Buenos Aires (ProgBA). The EQAS is accredited under ISO/IEC 17043:2010, and TSH is included in the scope. **Methods:** Freeze-dried human samples were distributed in 2017 to 200 participants in Latin America. Major IVD assays were IMMULITE SIEMENS, CENTAUR SIEMENS, ACCESS BECKMAN, COBAS ROCHE, and ARCHITECT ABBOTT, all of them calibrated against WHO IRP 80/558. Start-up materials were pooled sera or plasma from single donor coagulated with thrombin. Statistics were calculated following ISO 13528: 2015. All laboratories´ trimmed mean (ALTM) was used for comparison. **Results:** For low TSH level, ALTM=0.48 mUI/L, %differences ranged -10.03 to 15.7, span between lowest (COBAS) and highest (ACCESS) was 36%. For normal TSH levels, pooled serum ALTM=1.59 mUI/L %differences ranged -9.2 to 7.1, span between lowest (IMMULITE) to highest (COBAS) 16%; plasma ALTM 2.24 mUI/L %differences -12.6 to 5.1, span lowest (ARCHITECT) to highest (COBAS) 17%. For TSH level 4 mUI/L, plasma ALTM 4.41 mUI/L %differences -3.99 to 6.79, span lowest (ARCHITECT) to highest (CENTAUR) 11%. **Conclusion:** Results from EQAS showed same differences as those in the WG-STFT study, suggesting that differences observed are not defined by commutability of materials but by different assay standardization. Also these results support the role of EQAS as an important actor in IVD standardization / homogenization for hormone immunoassays.

## A-217

### New assay for testing anti-TSH receptor antibodies: is TSI comparable to other methods available?

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**Background:** Autoimmune thyroid illness (ATI) accounts for a number of disorders as hypothyroidism, hyperthyroidism and goiter. ATI is associated to autoantibodies against antigens like thyroid-stimulating hormone receptor (TSHR). Two types of TSHR antibodies are involved in the physiopathology of ATIs: 1) thyroid stimulating immunoglobulins (TSI) are present in Graves' disease (GD); 2) blocking antibodies prevent binding of TSH to its receptor (TRAb-B) and can lead to hypothyroidism in Hashimoto´ disease. Recently a third type was descripted, C-TRAb (Apoptotic), with biological activity, responsible together with cytotoxic Tcells specific for thyroid gland, of thyrocytes´ autoimmune depletion.Methods for TRAb detection include biological assays (BAs) and immunoassays (IAs). BAs measure TRAb activity while IAs measure antibody binding to receptor, without discrimination on function (totalTRAb). A new automated IA claims to detect only TSI, which would give high sensitivity in detecting GD, and high specificity for discriminating between GD and other ATIs. The aims of this work are the evaluation of performance of this new method for TSI and to compare it with other IAs available locally for TRAb testing. **Methods:** Immunoassays compared were TSI Immulite® (Siemens Healthcare), cutoff 0.55 IU/L calibration 2nd IS (NIBSC 08/204); TRAb% RSR® Limited (UK): cutoff 15%, calibration CRM LATS standard B; and TRAb-Cobas (Roche Diagnostics): cutoff 1.75 IU/L, calibration 1st IS (NIBSC 90/672). 290 negative, mild positive and strong positive serum samples from patients aged 6 to 82, 80% female, were included in the comparison: 148 samples were selected previously tested by RSR method and 142 by Cobas. 98 samples were tested by the three methods and the ratio of positive sample/cutoff (RP) was calculated for each method. **Results:** Comparing RSR and TSI (n=148), 24% samples were negative by both methods, 11.4% were discordant, and for those classified as positive by both methods, important differences were observed in RP values; when comparing TRAb-Cobas with TSI (n=142), 33% samples were negative by

the two methods, 7.7% samples were discordant, and for those classified as positive by the two methods, also important differences were observed in the RP values. For the 98 samples processed by the 3 methods, those showing values of 16-30% in RSR, in Cobas were between 0.90 y 18.87 IU/L and in TSI from 0.10 to 13.6 IU/L. Significant differences in the absolute values of RSR vs TSI and Cobas vs TSI were obtained (*p* less than 0.001, Wilcoxon signed Rank test, paired samples). Same differences were obtained comparing only positive samples in Cobas vs TSI (n: 89). Also, significant differences in RP values between RSR vs TSI and RSR vs Cobas (*p* less than 0.001) were obtained, but no significant difference was obtained between RP from TSI vs RP from Cobas (Friedman-Dunn test). **Conclusion:** Although the new Immulite method claims to be more specific for detection of TSI, the %discordances actually observed do not support this affirmation, as RP values do not correlate. We conclude that more data are needed to establish the real clinical usefulness of this new assay.

## A-218

### Performance evaluation of two fully-automated anti-Müllerian hormone assays and comparison against current manual enzyme-linked immunosorbent assay

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**Background:** Anti-Müllerian hormone (AMH), a dimeric glycoprotein produced by ovarian granulosa cells of pre-antral and small antral follicles, is a reliable serum biomarker to assess growing ovarian pool. AMH test is increasingly being used as a surrogate biomarker for ovarian reserve tests (ORTs) and recombinant follicle-stimulating hormone (rFSH) dosing in infertility clinics. However, its routine clinical use is limited by the low throughput and a high degree of inter-laboratory variability of enzyme-linked immunosorbent assays (ELISA). The authors evaluated the performance of two new fully automated AMH immunoassays and compared them with a currently used AMH ELISA method. **Methods:** Two fully-automated AMH assays with electro-chemiluminescence immunoassay (ECLIA) platform using the Roche cobas e602 analyzer (Roche Diagnostics GmbH, Germany) and chemiluminescence immunoassay (CLIA) platform using the UniCel DxI automated analyzer (Beckman Coulter Inc., USA) were compared to a current ELISA assay (AMH Gen II ELISA, Beckman Coulter, USA). Precision analysis (according to CLSI EP5 guideline), repeatability and linearity (according to CLSI EP6 guideline) were assessed for both of the two automated immunoassays. Anonymized remnant 113 serum samples from routine AMH ELISA testing were used for comparing the two automated immunoassays against the ELISA assay. **Results:** Both of the two automated AMH assays showed excellent precision, repeatability and linearity. The total coefficient variation (CV) of Beckman CLIA and Roche ECLIA assays were 3.5 - 4.1 % and 2.4 - 3.6 %, respectively, over a range of concentrations. AMH concentrations measured with the Beckman CLIA showed better correlation when compared with the AMH Gen II ELISA than the Roche ECLIA (CLIA = -0.149 + 0.877 ELISA, R = 0.983; ECLIA = 0.172 + 0.667 ELISA, R = 0.932). Both of the two automated assays showed significant negative bias when compared with the Gen II ELISA (mean % difference; -20.33% between ELISA and ECLIA vs. -12.17% between ELISA and CLIA). **Conclusion:** The two automated AMH immunoassays showed advantages including high throughput and superior analytical performance over the current Gen II ELISA platform. However, considerable degrees of systematic difference were noted between the automated immunoassays and the Gen II ELISA, with larger negative bias in case of the Roche ECLIA platform. Automated AMH assays need to be harmonized to the conventional ELISA to adapt currently using clinical cut-offs which is mostly based on the Gen II ELISA format.

## A-219

### The comparison of cortisol level by electrochemiluminescence Immunoassay radioimmunoassay and mass spectrometry

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**Background:** Cortisol is the main glucocorticoid of the adrenal cortex, regulating energy metabolism, blood pressure, stress responses, etc. To diagnose cortisol related disease such as Cushing's syndrome or Addison's disease, it is important to provide accurate level of cortisol. In Korea, current practice to measure cortisol level is to use electrochemiluminescence Immunoassay(ECLIA) and radioimmunoassay(RIA). Immunoassays have problems with varying selectivity and specificity. As for cortisol, there are many endogenous metabolites and drugs that interfere with immunoassay. In this study, we compared HPLC-MS/MS with ECLIA and RIA to measure serum cortisol level. **Methods:** Cortisol levels from 28 samples referred to Seoul Clinical Laboratories(SCL) in Korea were measured by MS/MS(AB Sciex, CA, USA), ECLIA and RIA. 200ul samples, control, and calibrator with cortisol-9,11,12,12-d4 were mixed with ethyl acetate 2mL. After 30 seconds of vortexing, centrifugation for 5 minutes at 4000 rpm was performed. Obtained supernatant was placed under, N2 gas for evaporation. Reconstruction was done with 100uL of 30% methanol. 20 uL of sample and control were placed on auto-sampler. Separation was carried out on Imtakt Unison UK C18 column(2.0 x 50 mm, 3um) followed by MS detection using an ABSciex 5500 mass spectrometer. Detection was performed in the positive electrospray multiple reaction monitoring (MRM) quantitation. ECLIA of cortisol was performed on the Cobas e601(Roche, Grenzacherstrasse, Switzerland). The RIA(Immunotech s.r.o, Prague, Czech) was performed according to the manufacturer's instructions. Samples and calibrators are incubated with an 125I-labeled cortisol, as tracer, in antibody-coated tubes. Results were compared by ANOVA, linear regression analysis and using the methods described by Bland and Altman. **Results:**
• Cortisol levels by MS/MS, ECLIA and RIA ranged from 1.35 to 48.8 (median 20.98) ug/dL, from 0.99 to 55.2 (median 21.18)ug/dL and from 1.20 to 68.3 (median 23.89)ug/dL, respectively. ANOVA analysis concluded three methods do not show difference. • Numbers of samples higher than the reference value by MS/MS, ECLIA and RIA were 11, 12 and 11, respectively. • Numbers of samples lower than the reference value by MS/MS, ECLIA and RIA were 4, 6 and 3, respectively. • When comparing MS/MS with ECLIA, linear regression analysis revealed the following equation: ECLIA = 1.0999xMS/MS - 1.8929 ($R^2$ = 0.9821). • When comparing MS/MS with RIA, linear regression analysis revealed the following equation: RIA = 1.2677xMS/MS - 2.7024 ($R^2$ = 0.8891). **Conclusion:** Although ANOVA analysis concluded three methods do not show difference, correlation between RIA and MS/MS was not as good as ECLIA and MS/MS. The difference between RIA and MS/MS gets bigger as the level of cortisol goes higher, which becomes as big as -22.10ug/dL. The external quality controls of three methods have been within acceptable ranges. Therefore, it is recommended for clinicians to recognize different method can show different result. Furthermore, there should be international effort to develop certified reference materials(CRM) and accuracy based proficiency test to standardize the measurement of cortisol.

## A-220

### Development of the ARCHITECT Tg Assay for Quantitation of Human Thyroglobulin

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**INTRODUCTION:** Thyroglobulin (Tg) is a protein that is readily secreted by the thyroid gland. Tg is primarily used as an aid in monitoring after total thyroid ablation (thyroidectomy). **METHODS:** The ARCHITECT Tg assay (in development) is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative measurement of Tg in human serum or plasma on the ARCHITECT *i* System. This method utilizes paramagnetic microparticles coated with a highly specific monoclonal antibody (mAb), which captures Tg present in the specimen. After incubation, the acridinium-labeled anti-Tg mAb conjugate is added to complete the sandwich. After another incubation and wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of Tg present in the specimen and the RLUs detected by the ARCHITECT *i* System optics. **RESULTS:** The ARCHITECT Tg assay is traceable to the European Community Bureau of Reference (BCR) CRM 457. Across three lots, the ARCHITECT Tg assay demonstrated linearity from 0.05-673.72 ng/ml. The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the ARCHITECT Tg assay were 0.06 ng/mL, 0.10, ng/ml. and 0.12 ng/ml, respectively. A twenty-day precision study of 3 controls and 5 panels assayed twice per day (n=80 for each sample) demonstrated within laboratory (total) precision of ≤ 5.5% for controls and ≤ 4.9% for panels. A method comparison of the ARCHITECT Tg assay with a predicate device was performed using Passing-Bablok regression resulted in a 0.99 slope, 0.31 ng/mL y-intercept, and 0.995 correlation coefficient (r) across the assay range of 0 - 500 ng/mL. In the range of 0 -10 ng/mL, Passing-Bablok regression resulted in a 1.06 slope, 0.00 ng/mL intercept, and 0.97 correlation. A tube-type equivalence study was performed using 6 non-primary tubes (Plasma K2 EDTA, Plasma K3 EDTA, Sodium EDTA, Serum Separator, Plasma Lithium Heparin, and Plasma Separator) compared directly to a primary Serum red-top with observed slopes of 0.96-1.01. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfer-

ing compounds, including 7 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum album, human anti-mouse antibody, and rheumatoid factor) and 14 commonly used therapeutic drugs (aminodarone HCL, carbimazole, D-T3, hydrocortisone, iodide, L-T3, L-T4, octreotide, potassium percholorate, prednisolone, popanolol HCL, propylthiouracil, thiamazole, fluocortolone pivalate). Cross-reactivity of the ARCHITECT Tg assay with Thyroid-Stimulating Hormone (TSH, 1000 mIU/L) and Thyroxine-Binding Globulin (TBG, 200,000 ng/ml) that are both similar in structure to Tg demonstrated no detectable cross-reactivity (0%). There was no high-dose hook effect observed for samples containing up to ~100,000 ng/ml of Tg. The ARCHITECT Tg reagents demonstrated on board stability and calibration stability on the instrument for a up to 30 days. **CONCLUSIONS:** These data demonstrate that the ARCHITECT Tg assay is sensitive, accurate and precise for the quantitative determination of Tg in serum and plasma specimens.

## A-221

### Performance of the Diazyme Laboratories, Inc Glycated Serum Protein (Glycated Albumin) Assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System.

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**Background:** The Diazyme Laboratories, Inc. glycated serum protein (GSP) assay quantitatively determines the concentration of glycated proteins (Glycated Albumin) in serum. GSP is formed by a non-enzymatic Maillard reaction between glucose and amino acid residues of proteins. In diabetic patients, glycated serum proteins are a medium term indicator of diabetic control (2-3 weeks). GSP can be used in patients with conditions that interfere with RBC lifespan which may reduce the reliability of HbA1c measurements. Traditional fructosamine is also a glycated protein based assay but it utilizes a nitroblue tetrazolium (NBT) method, which in addition to glycated proteins reacts with various endogenous reducing substances such as thiol groups, NADH, and ascorbate. Studies showed that only about half of the reducing activity (Fructosamine) was due to glycation of proteins, and the remaining unspecific activity varied from serum to serum. The mechanism for the Diazyme GSP assay is that GSP is enzymatically digested and a proprietary amadoriase is used to catalyze the oxidative degradation of Amadori product protein and amino acids to glucosone and $H_2O_2$. The $H_2O_2$ released is measured by a colorimetric Trinder end-point reaction at 540 nm which is proportional to the concentration of glycated serum proteins. **Methods:** . The performance of the Diazyme GSP assay on the VITROS® 4600 Chemistry System and the VITROS® 5600 Integrated System was assessed on the VITROS MicroTip assay processing side of the MicroImmunoassay Center using 7.5 uL of patient sample and the Diazyme GSP reagents. Endpoint absorbance measurements were converted to a concentration using a linear calibration model. **Results:** The accuracy of Diazyme GSP assay was evaluated with 65 serum patient samples (134 - 1230 µmol/L) on the VITROS 4600 and VITROS 5600 Systems then compared to the predicate device Roche Hitachi 917 following CLSI: EP9-A2 guidelines. The VITROS 4600 and VITROS 5600 System showed excellent correlation with the Roche Hitachi 917. VITROS 4600 System $R^2$ value of 0.9946 with a slope= 1.0024, y-intercept of -4.77 and % bias of ≤ 10%. VITROS 5600 System $R^2$ value of 0.9911 with a slope= 1.0182, y-intercept of -3.07 and % bias of ≤ 10%. A 20-day precision study conducted on the VITROS 4600 and VITROS 5600 Systems at mean GSP concentrations of 251 µmol/L and 743 µmol/L resulted in within-laboratory percent coefficient of variation (%CV) of 3.76 % and 3.11% respectively, for the VITROS 4600 System and 2.40% and 1.55% respectively, for the VITROS 5600 System. The Limit of Quantification (LoQ) check for the VITROS 4600 and VITROS 5600 Systems was found to be ≤15.0 µmol/L. At 300 µmol/L common interfering endogenous substances of ascorbic acid 5 mg/dL, bilirubin 7.5 mg/dL, conjugated bilirubin 5 mg/dL, hemoglobin 200 mg/dL and triglycerides 2000 mg/dL showed no significant interference (≤ 10%). **Conclusion:** The Diazyme Glycated Serum Protein assay run on the VITROS 4600 and VITROS 5600 Systems demonstrated excellent correlation with the Roche Hitachi 917 Clinical Chemistry Analyzer, exceptional precision, and low-end sensitivity. Additionally, the assay was free from interference by endogenous substances at clinically relevant GSP concentrations.

## A-222

### Dihydrotestosterone (DHT) Quantification in Blood Serum by LC-MS/MS after Derivatization for Research Purposes

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**Background:** Reduction of testosterone by 5α-reductase yields dihydrotestosterone (DHT), which is a more potent androgen than testosterone. Researchers studying the physiology of DHT and control of its biosynthesis using 5α-reductase inhibitors need to quantify this steroid within a range of 25 to 2000 pg/mL (2.5 - 200 ng/dL or 0.09 to 6.88 nmol/L) in blood serum. Since DHT does not ionize well by either atmospheric-pressure chemical ionization (APCI) or electrospray ionization (ESI), derivatization with hydroxylamine prior to liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) was necessary to reliably achieve the desired measuring range. **Methods:** DHT was measured in donor blood serum samples using a multi-channel LC system coupled to a triple-quadrupole mass spectrometer with heated ESI source. 200 µL aliquots of specimens (calibrators, quality controls and donor serum samples) were spiked with DHT-D3 internal standard (IS) and then extracted by 2 mL of methyl-tert-butyl ether. The extracts were evaporated and the residues were reacted with hydroxylamine to form positive-ion oxime derivatives. The preparations were dried and reconstituted with 200 µL of water and methanol (1:1). 50 µL injections were made into heated 100 x 2.1 mm columns packed with solid-core silica particles with C18 and polar end caps bonded to available surfaces. A 4.5-minute mobile phase gradient from 50% methanol in water containing 0.1% formic acid to 100% methanol separated and eluted DHT and IS into the heated ESI source of the MS/MS system. Selected-reaction monitoring (SRM) of two transitions (quantitation and conformation) for DHT and IS occurred within a 2-minute data window. **Results:** Confirmation/quantitation ion ratios among calibrators, QCs, and specimens were within 20% of averages calculated from the calibrators. Intra-and inter-batch precisions among 20 replicate injections from three pools (low, medium and high DHT levels) were less than 7%CV. Carryover never exceeded 0.2%. Specimen IS peak areas averaged 70% relative to the averaged IS peak areas in calibrators and QCs, indicating moderate ion-suppression by matrix. However, the IS in each sample adequately compensated for matrix effects. The desired measuring range from 25 to 2000 pg/mL was achieved and was consistently linear ($r2 \geq 0.995$ with 1/X weighting). 54 donor samples were analyzed and results were compared with those from a reference lab. DHT values ranged from 47 to 973 pg/mL. The two DHT methods were equivalent within an allowable total error (TEa) of 25%. Only 4 out of 54 results differed by more than 20% and none were more than 22.4%. The differences between the two methods averaged 2.5%, which is a small positive bias. **Conclusion:** Derivatization with hydroxylamine prior to LC-MS/MS permitted reliable quantification of DHT in serum between 25-2000 pg/mL at a throughput of 13 injections/hour on a single LC channel. Throughput doubled by using an additional LC channel.

## A-223

### Causes of Calculation Error Messages Encountered During the Measurement of HbA1c Using Cobas c502 Tina-quant® Assay

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**Background:** The Tina-quant Hemoglobin A1c (HbA1c) assay is a test with high specificity that can be used to diagnose diabetes, identify people at risk of developing the disease and ongoing monitoring. The aim of this study was to evaluate the possible causes of calculation error messages (CEMs) encountered during the measurement of HbA1c using this assay. **Methods:** All HbA1c samples performed over a period of one month, using Cobas c502 turbidimetric inhibition immunoassay (Tina-quant HbA1c assay), and had CEMs were investigated according to a special flow chart for HbA1c flags constructed by our laboratory according to manufacturer's recommendations. The error messages were divided into 4 categories; HbA1c less than the detection limit (A1 < test), HB less than the detection limit (HB < test), HbA1c more than the detection limit (A1 > test) and HB more than the detection limit (HB > test). Samples with CEM were checked for the presence of clots, remixed properly by inversion 20 times and reran on Cobas c502. Samples that gave a CEM of error >test after remixing and repeating were retested after dilution with 0.9% saline. Hematology samples, collected on the same day and at the same time, were used to double check and/or confirm the results of some of the samples with CEM. **Results:** out of 17,600 HBA1c samples, 177 (1%) were associated with CEMs; 120 (67.4%) had A1 < test, 72 (40.4%) had HB < test, 54 (30.3%) had A1 > test and 13

(7.3%) had HB > test. Evaluation by applying our flow chart for HbA1c flags revealed the association between theses samples and different conditions; 116 (65.5%) had low or high CEMs and corrected after remixing, 3 (1.7%) had low CEMs with normal HB and did not correct after remixing, 15 (8.5%) had low CEMs with low HB and were cancelled, 7 (4.0%) had high CEMs and gave exact readings after dilution, 30 (16.9%) clotted samples and were cancelled and 6 (3.4%) which were kept at room temperature for long time, had high CEMs, but refrigerated hematology samples of the same patients directly gave exact readings without the need for dilution. Total number of canceled results was only 33 (18.6%) of the samples with CEMs. **Conclusion:** Majority (83.4%) of CEMs are caused by preanalytical errors, which can be effectively prevented by simple techniques such as proper mixing and refrigeration of samples.

## A-224

### Macroprolactin is not predicted by prolactin concentrations greater than 100 ug/L above validated prolactin reference intervals

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**Objective** Confirmation of current prolactin reference intervals in male and female populations, and identify their application in defining reflex testing rules for macroprolactin assessment in community and tertiary care patients. **Relevance** Prolactin is released from the anterior pituitary; it is the principle hormone controlling lactation initiation and maintenance. Hypo- and hyperprolactinemia may be associated with endocrine disorders, and hyperprolactinemia may also occur from prolactin secreting pituitary adenomas. Differential diagnosis of these conditions relies upon suitable prolactin reference interval, while also ruling out macroprolactin in asymptomatic patients. Macroprolactin is a prolactin complex bound to immunoglobulins, it is biologically inactive, and has a prolonged half-life versus monomeric prolactin. The macroprolactin complex is immunoreactive and can interfere in prolactin measurement, resulting in high prolactin concentrations despite asymptomatic patient presentation. **Methodology** Prolactin and macroprolactin results were compiled from community and tertiary care patients in Calgary, AB between January 1, 2015 and March 31, 2017 (*N*=65,561). Prolactin concentrations from plasma and serum specimens were measured using a sandwich immunoassay with two monoclonal antibodies against human prolactin and electrochemiluminescent detection (Roche Cobas 8000 e602). Macroprolactin testing was completed on all patients with prolactin concentrations above the upper reference limit (URL; 15 ng/mL men; 25 ng/mL women), with diagnosis of macroprolactin based upon prolactin recovery in polyethylene glycol (PEG) treated vs untreated specimens. A macroprolactin result with ≤40% recovery indicates macroprolactin (40-60% indeterminate). Prolactin reference intervals in both male and female populations were assessed by: 1) Bhattacharya analysis (*N*=15,529), 2) measurement in random specimens (*N*=60), and 3) measurement in healthy volunteers (*N*=20). Bhattacharya analysis and correlation assessment between macroprolactin incidence and prolactin concentration was performed in Microsoft Excel 2010 and IBM SPSS Statistics 19. **Results** Over approximately 2 years, macroprolactin testing increased (6.8-fold males; 1.8-fold females), while prolactin testing increased by 11.2% in males and decreased by 12.3% in females. Reference intervals were established (4-25 ng/mL females; 4-15 ng/mL males) following measurement in healthy volunteers. Positivity for macroprolactin was 4% in males and 7% in females, while indeterminate result was found in 4% of males and 10% of females. Prolactin concentration was identified as a poor predictor of macroprolactin detection by PEG precipitation; restricting macroprolactin measurement in specimens below the upper limit of the prolactin reference interval is a better predictor. In addition, prolactin results >100ng/mL above the URL (>116 µg/L males; >126 µg/L females) should not undergo macroprolactin investigation, as they have 0% positive predictive value for macroprolactin (N=569). **Conclusions** Prolactin reference intervals can only be determined within correctly identified healthy populations due to significant variability and preanalytical influences. Prolactin concentration was a poor predictor of macroprolactin findings; macroprolactin was not found in any specimens with prolactin concentrations >100 µg/L above the upper reference interval.

## A-225

### Performance Evaluation of a Coupled Enzymatic Glycated Albumin Assay in Serum and Plasma Samples

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**Background:** Glycated albumin is a plasma protein formed by the nonenzymatic reaction of glucose and albumin. It has utility in monitoring glucose control in patients for whom hemoglobin A1c is considered unreliable. This includes those in whom the erythrocyte life span is altered, patients undergoing dialysis for end stage renal disease, or women during pregnancy. Additionally, it provides information on short-term (2-4 weeks) glycemic control that could be used prognostically. Although widely used in Japan, FDA-approved tests for this analyte are lacking in the U.S. We thus examined the performance of a commercially-available glycated serum protein assay which utilizes a coupled enzymatic assay adaptable for use on a variety of automated chemistry analyzers. **Methods:** Samples used in evaluation were either serum or plasma (heparin- or EDTA-containing tubes). Glycated Serum Protein (µmol/L, GSP) was measured using a coupled enzymatic assay (Diazyme, Poway, CA) on a Roche Cobas c501 automated platform. Albumin was measured using the Roche bromocresol green assay on the same platform. Percent glycated albumin (% GA) was calculated from GSP and albumin concentrations using the equation recommended by the manufacturer. Multi-day and within-day imprecision were determined using percent coefficient of variation (%CV). Accuracy was examined by comparison to certified reference material (JCCRM611-1) and samples obtained from the College of American Pathologists Proficiency Testing (CAP-PT) Survey. Linearity was examined using eight non-zero points in duplicate. **Results:** For serum, typical within-day %CV was 1.6% at both 13.0% and 50.2% GA. For heparin plasma samples, within-day %CV was 2.3% at 18.8% GA and 1.7% at 13.3% GA. %CV was slightly higher for the GSP measurement alone. Between-day precision (7 days, triplicates) was 2.3% for 13.6% GA in serum; in heparin plasma, it was 2.5% for 15.0% GA and 3.3% for 26.7% GA. Percent recovery compared to JCCRM611-1 was 103-104% at 12.8% and 20.2% GA. Results also fell within the expected range compared to peer results from the CAP-PT Survey. Linearity and lower limit of quantitation showed acceptable results for the GSP range of 86 -946 µmol/L (m= 1.014, b= -1.65, standard error of the estimate=14.74). With an albumin of 4.4 g/dL this range would correspond to 7.0 - 43.0% GA. A previously published reference interval of 10.5 - 17.5% (J Diabetes Sci Technol 2015; 9:192-9) was verified using 50 patient samples (25 female, median age 43 years, HbA1c ≤5.7), with only one outlier. Serum samples at room temperature were stable for 6 hours (<1.1% difference). Plasma samples collected in heparin tubes showed <4% difference in results under these conditions. Both matrix types showed stability to 7 days at 2-8 °C, but were no longer stable at day 14. Samples collected in EDTA showed instability during the same conditions. **Conclusion:** This assay for %GA shows very good precision and accuracy at low and high concentrations of analyte. The linear measuring range as determined for GSP, when using an average albumin concentration, corresponds to 7.0% - 43.2% GA, which would be useful for diabetic patients. Both heparin-containing and serum tubes are acceptable.

## A-226

### Creating highly accurate and precise measurements of free thyroxine (T4) for the CDC Clinical Standardization Programs

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**Background:** Free thyroxine (FT4) measurements are used to diagnose and treat thyroid disorders, such as Graves' disease, Hashimoto's thyroiditis, and thyroid cancer. Reliable FT4 measurements are essential for assessing thyroid function and properly diagnosing and treating thyroid disorders. Although FT4 measurements are used extensively in research and clinical settings, the accuracy and reliability of current methods prevent proper detection, treatment, and prevention of thyroid disorders in patient care, making standardization of FT4 measurements a priority. Currently, there are no serum-based reference materials commercially available for FT4. CDC is working with the Committee for Standardization of Thyroid Function Tests (C-STFT) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to address these issues through development of an accurate and sensitive higher-order Reference Measurement Procedure (RMP) for FT4 as part of IFCC C-STFT and CDC's Clinical Standardization Programs. **Methods:** FT4 was measured using a modified equilibrium dialysis procedure.[1] FT4 in serum was isolated from the binding proteins in 1 mL PTFE equilibrium dialysis cells for 4 hours at 37°C. Dialysates were spiked with internal standard (thyroxine-13C6) and purified by C18 solid phase extraction (SPE) prior to injection on a Shimadzu UHPLC system coupled with an AB Sciex 5500 triple quadrupole mass spectrometer equipped with a TurboV electrospray ionization source. Bracketed calibration and primary reference materials were used to determine concentration of FT4 in serum. Chromatographic separation was achieved using a C18 reverse phase column with a gradient of water and acetonitrile with addition of 0.1% formic acid. Quantification by selective reaction monitoring (SRM) analysis was performed in the positive ion mode. Two transitions were monitored for each analyte and internal standard, and triplicate injections were used to minimize any instrument instability. **Results:** The proposed RMP has been evaluated and optimized for precision, accuracy, and sensitivity. The within-day and between-day impreci-

sion of 2.2-3.9% and 1.8-2.6%, respectively, were determined using CLSI EP10. By comparisons with the Reference Laboratory at the University of Ghent, the proposed CDC RMP reported a bias within +1.0%. Maximum extraction of the analyte prior to injection on the mass spectrometer is critical to ensure the sensitivity of the method is adequate. For example, it was determined that a 55% loss in signal could occur during sample preparation if the sample came into contact with either PTFE-lined or rubber caps, and a 16.5% loss in signal could occur with the use of plastic wellplates versus glass LC/MS vials before analysis. Taking into account these key factors, the limit of detection using 1.0 mL of serum was 0.312 pg on-column (0.126 ng/dL). **Conclusion:** To ensure accurate values are reported for FT4, careful consideration is needed for all steps of sample preparation and analysis. This candidate reference method for FT4 in serum demonstrates good accuracy and precision, and as such this method can be used as a viable base for accuracy to which routine methods for FT4 can be compared. [1]Van Houcke, A.K., et. al. IFCC international conventional reference procedure for the measurement of free thyroxine in serum. *Clin. Chem. Lab. Med.* 2011, 49, 1275-1281.

## A-227

### Establishment of reference interval for thyroid stimulating hormone using the Bootstrap analysis and strict reference individual selection criteria from the Korean nationwide data

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**Background:** Serum thyroid stimulating hormone (TSH) level was influenced by several factors, including age, gender, smoking and intake of dietary iodine. We evaluated several factors specific reference interval of serum TSH levels using the Bootstrap analysis and strict reference individual selection criteria from the Korean nationwide data, a country known to be an iodine uptake excess area and **Methods**: The sixth Korean National Health and Nutrition Examination Survey (2013-2015) is a nationwide, cross-sectional survey of the Korean general population. Initially, a total of 6,905 participants aged over 10 years who underwent TSH and urinary iodine level measurements were selected. 2,582 participants who had any cases of acute or chronic disease and those taking the drug as related diseases in the health questionnaire data and 2,455 participants whose another laboratory results were abnormal were excluded. After excluding 127 statistical outlier cases, the 1,741 participants were included. TSH and urinary iodine (UI) levels were measeured by an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany) and inductively coupled plasma mass spectrometry PerkinElmer, Waltham, MA), respectively. Analyse-it v 4.80 was used for Bootstrap calculation of reference interval. **Results:** The reference interval of TSH was 0.568-4.916 mIU/mL. Factor specific TSH reference interval is shown in Table 1. With the increase of UI, higher reference limit of TSH tended to increase. **Conclusions:** Strict criteria for healthy population is essential for establishing reference intervals and accurate assessment of thyroid function. We used the nationwide survey and exclusion criteria for selection of healthy to demonstrate Korean TSH reference interval. Nutritional iodine status might need to be more useful to establishing TSH reference intervals of populations in iodine -replete areas.

Table 1. Reference Interval of Serum TSH According to Gender, Age, Body Mass Index, Smoking Status and

| Population | | | N | Reference interval of serum TSH (mIU/L) | |
|---|---|---|---|---|---|
| | | | | Lower limit (90% CI) | Higher limit (9% CI) |
| Total | | | 1,741 | 0.568 (0.480-0.630) | 4.916 (4.764-5.0690) |
| | Gender | Male | 1,021 | 0.578 (0.500-0.670) | 4.981 (4.794-5.110) |
| | | Femal | 720 | 0.534 (0.431-0.680) | 4.821 (4.619-5.050) |
| | Age | 10-19 | 615 | 0.688 (0.570-0.800) | 5.121 (4.970-5.294) |
| | | 20-29 | 342 | 0.582 (0.409-0.728) | 4.883 (4.580-5.160) |
| | | 30-39 | 315 | 0.532 (0.321-0.730) | 4.635 (4.431-4.940) |
| | | 40-49 | 225 | 0.621 (0.316-0.776) | 4.485 (4.177-4.920) |
| | | 50-59 | 157 | 0.214 (0.010-0.484) | 4.704 (4.330-5.210) |
| | | ≥ 60 | 87 | 0.362 (0.110-0.566) | 4.872 (4.158-5.500) |
| | Body Mass Index | <18.5 | 208 | 0.584 (0.120-0.888) | 5.205 (4.910-5.400) |
| | | 18.5-24.9 | 1,129 | 0.563 (0.480-0.680) | 4.951 (4.780-5.090) |
| | | 25-29.9 | 299 | 0.480 (0.310-0.610) | 4.670 (4.355-4.990) |
| | | ≥ 30 | 105 | 0.620 (0.260-0.850) | 4.428 (4.111-4.590) |
| | Current smoking | No | 1,467 | 0.576 (0.480-0.658) | 5.006 (4.855-5.110) |
| | | Yes | 274 | 0.507 (0.425-0.627) | 4.012 (3.920-4.180) |
| | Urine iodine (μg/L) | < 208.8 | 434 | 0.557 (0.409-0.684) | 4.526 (4.363-4.671) |
| | | 208.8-364.0 | 436 | 0.751 (0.619-0.830) | 4.981 (4.599-5.224) |
| | | 364.1-837.1 | 435 | 0.512 (0.377-0.673) | 5.001 (4.801-5.272) |
| | | ≥ 837.2 | 436 | 0.447 (0.300-0.539) | 5.038 (4.741-5.132) |

## A-228

### New genetic variants of hypophosphatasia. Retrospective study of hypophosphatasia in Granada, Spain

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**Background:** Hypophosphatasia (HPP) is a rare, serious and potentially mortal genetic disease caused by one or several mutations in the gene coding for the alkaline phosphatase without tissue specificity (TNSALP). A low serum total alkaline phosphatase (ALP) level, associated with clinical and radiographic findings, is the hallmark for the diagnosis of HPP. The childhood form of the disease is characterized by rickets-related deformities of the skeleton, craniosynostosis, delays in walking, short stature, fractures and bone pain, and early teeth loss. The diagnosis of adult forms of HPP is a challenge; patients typically present in middle age with recurrent poorly healing metatarsal stress fractures or atypical diaphyseal subtrochanteric femoral fracture; they have also an increased risk of chondrocalcinosis, enthesopathies with ossifications, and even calcific periarthritis. **Objective:** To assess the recognition of persistent low ALP levels in a tertiary care hospital in Granada to identify the patients affected with HPP, to provide an appropriate management avoiding potentially harmful drugs as antiresortive treatments. **Methods:** Between 1st of January and 31st of December 2016, 78590 patients had ALP assessment in the Biochemistry Department of our hospital. The database was divided into adult population and pediatric population. Ninety-eight patients (66 adults and 32 children) had several serum ALP values persistently below the reference interval (30 IU/l for adults, 100 IU/l for children 0-12 years old and 50 IU/l for children 13-19 years old). Through summary discharges consulting, 22 patients were discarded because of potential causes of secondary HPP. Twenty-four potential HPP patients were contacted to fulfill a questionnaire about clinical manifestations potentially related to HPP. We sampled with EDTA total blood these patients for the determination of pyridoxal-5'-phosphate (PLP) that was determined by high-performance liquid chromatography (HPLC), as well as amplification and subsequent sequencing by PCR of the coding regions and exon-intron junctions of the ALPL gene, using as reference the truncated NM_000478.4. The variants found were validated with a PCR repeat and the altered sequences. This project was approved by Ethical Committee from Andalucía Biomedical research. **Results:** 0.12% of patients who had routinaire biochemical tests along 2016 in Granada, had persistently low value of ALP. Twenty-four patients were contacted. Among them, 10 patients had fractures; 4 had symptomatic chondrocalcinosis and 4 had dental abnormalities. In all of them, ALP and PLP levels were determined and genetic analysis was performed. Nine adults and 2 children presented decreased ALP and increased PLP levels compared to the reference interval. From these 11 patients, 7 adult patients presented TNSALP mutations, 4 of them corresponded to new pathogenic variants not described previously. **Conclusions:** The study supports the effectiveness of performing screening with persistent low levels of ALP to detect cases of PPH. Our study shows that there are several omitted diagnostics of HPP in a tertiary care hospital. From 78590 patients analyzed, 7 of them presented TNSALP mutation obtaining 4 new genetic variants. These data indicate 9/100000 of HPP prevalence in Granada.

## A-229

### Spironolactone metabolite causes falsely increased progesterone in the Abbott Architect Immunoassay

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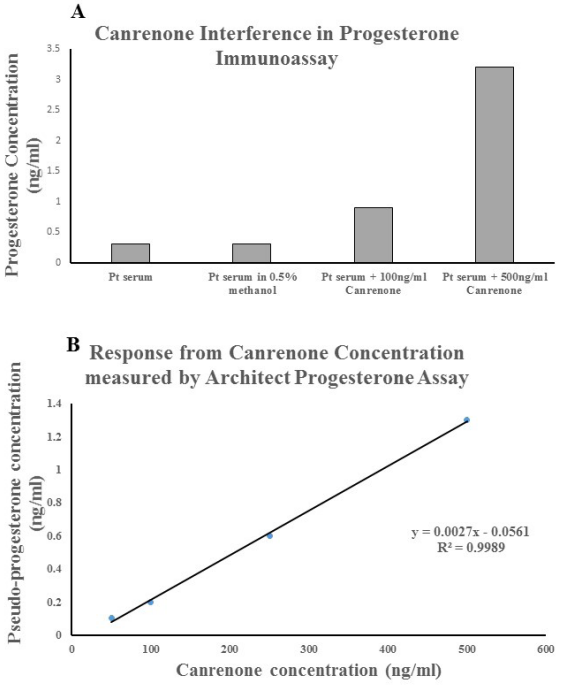
**Background:** Clinical laboratory determination of progesterone is a critical component for evaluating women for infertility. Primary hirsutism and anovulation are typically treated with spironolactone, a potent androgen-receptor antagonist. We identified discrepant serum progesterone measurements in patients who were prescribed spironolactone when measured on the Abbott Architect compared to the Siemens Immulite. Although spironolactone and its clinically active metabolite (canrenone) are structurally similar to progesterone, no current studies have elucidated the interference of this medication or its active metabolites in immunoassay-based progesterone measurements. **Method:** Progesterone concentrations were compared on two different immu-

noassay analyzer platforms, Abbott Architect i2000 and Siemens Immulite 2000 XPi. To investigate spironolactone and canrenone as possible interferants, we designed interference studies with commercially available material as recommended by the CLSI EP7-A2 clinical laboratory guideline. Sprinolactone or canrenone (at physiologic concentrations) was spiked in serum and analyzed on the Architect. **Results:** Patients on spironolactone showed increased progesterone concentrations (4 - 7 relative fold change) on the Architect system compared with concentrations obtained using the Immulite. Additional spiking studies with the parent drug, spironolactone, showed no interference. However, spiking canrenone into serum, at concentrations of 100 ng/mL and 500 ng/mL respectively, resulted in a 2- to 10-fold increase in progesterone when measured on the Architect (Fig. A). To further investigate the cross-reactivity of canrenone in the progesterone assay, increasing concentrations of canrenone was added into the assay-specific diluent. This produced a linear response with a positive slope on the Architect (Fig. B). **Conclusion:** The data shows a linear relationship between the concentration of canrenone and progesterone measured by the Architect assay. We report, for the first time that canrenone, a metabolite of spironolactone interferes with the Architect progesterone assay and falsely increases the results.




## A-230

### Utility of thyroid function reflexive testing in inpatients with comorbidities

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**Background**: The American Thyroid Association, American Association of Clinical Endocrinology and National Academy of Clinical Biochemistry committees have all recommended a thyroid-stimulating hormone (TSH)-first algorithm for screening of thyroid function disorders. Simultaneous testing of TSH and free thyroxine (FT4) is recommended only for patients with unstable thyroid status, such as during the first 2-3 months of treatment for hypo- or hyperthyroidism, in hypothyroid patients suspected of non-compliance with levothyroxine replacement, and in acute conditions. However, in managing patients with comorbidities, many physicians still order TSH with FT4 due to concerns of sick euthyroid syndrome, or unstable thyroid hormone balance. This ordering pattern may lead to unnecessary testings. The study objective was to investigate if a thyroid function testing algorithm with TSH reflexed to FT4 could be applied to patients with comorbidities in inpatient settings. **Methods**: We randomly selected 100 pairs of TSH/FT4 test results at Texas Children's Hospital in-patient laboratory from September to December 2017. FT4 was measured on Vitros 5600 (Ortho Diagnostics), and TSH was measured on Architect i1000SR (Abbott Diagnostics). Patients' medical records were reviewed by clinical chemists and were grouped based on screening or monitoring purposes of the test orders, and based on physician specialty. The screening scenario was defined as no previously identified thyroid or pituitary disorders in the patient, and monitoring scenario was defined as known histories of thyroid or pituitary disorders, or histories of abnormal thyroid test results in patients. **Results**: Out of the 100 paired TSH/FT4 orders, there were 34 orders for monitoring purpose, which included 9 patients on thyroid hormone replacement or TSH inhibition therapy. The rest of the 66 orders were for screening thyroid function purposes. Among the 66 screening orders, 16 were from patients presenting to the emergency department, 9 from endocrinologists, 8 from critical care units, 8 from hematology-oncologists, and 3 as part of pre-operative testing. More importantly, of the 100 TSH/FT4 pairs, 67 showed TSH and FT4 levels both within the reference interval (RI) and 29 had an abnormal TSH and only 5 also had an abnormal FT4. Only 4 pairs had a normal TSH and abnormal FT4 and would not have been captured in a TSH first reflexive algorithm. Of these 4 pairs, two fell into the monitoring group where 1 patient was a pregnant woman with diagnosed Graves' disease, and the other patient was diagnosed with panhypopituitarism. The other two pairs of TSH/FT4 screening orders were from a patient with microcephaly, hypothermia and a patient with septicemia, both in critically ill state. **Conclusion**: This study of 100 paired in-patient TSH/FT4 orders revealed that an algorithm of TSH reflexed to FT4 would be able to capture the majority (96%) of thyroid function disorders in patients with multiple comorbidities. This would result in effective utilization of thyroid function tests and decrease test costs for the patient. Simultaneous measurement of TSH and FT4 should not be encouraged for thyroid function disorder screening purposes, except in patients in acute care setting, or when there is indication of unstable thyroid status.

## A-231

### Evaluation of plasma adropin levels and cardiometabolic risk indices in type 2 diabetic patients in south west nigeria

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**Introduction:** Diabetes mellitus is a group of metabolic disorders characterised by dyslipidaemia and hyperglycaemia due to reduced insulin secretion or insulin resistance. Adropin as a peptide hormone has been reported to promote insulin sensitivity and secretion. **Objective:** This study was designed to evaluate plasma adropin levels in type 2 diabetic patients in South-West, Nigeria **Research Design and Methodology:** A total of 130 patients (37 males, 93 females) with age range of 42-70 years diagnosed as type 2 diabetes mellitus, were recruited for this study. Forty three apparently healthy volunteers (16 males, 27 females) with age range of 30-55 years, were included as controls. Adropin hormone level was determined by ELISA technique. Fasting plasma glucose, lipids, lipoproteins and anthropometric indices were determined using standard procedures. **Results:** The results showed significant increases in waist circumference, hip circumference, pulse rate ($p = 0.000$), waist to hip ratio ($p = 0.006$) and systolic blood pressure ($p = 0.05$) when compared with the control values. Conversely, there were significant decreases in height ($p = 0.005$) and diastolic blood pressure ($p = 0.004$) when compared with control values. There were also significant increases in fasting plasma glucose, total cholesterol, triglyceride, low density lipoprotein cholesterol/high density lipoprotein cholesterol, total cholesterol/high density lipoprotein cholesterol ratio ($p = 0.000$) when compared with the control values; while there was significant decrease in high density lipoprotein cholesterol when compared with the control values. There was a remarkable significant decrease in adropin ($p = 0.000$) when compared with the control values. **Conclusion:** The results from the present study provide evidence that decreased levels of adropin, high density lipoprotein cholesterol coupled with increased levels of fasting plasma glucose, total cholesterol, triglyceride, low density lipoprotein cholesterol/high density lipoprotein cholesterol, total cholesterol/high density lipoprotein cholesterol ratio are the main biochemical changes associated with type 2 diabetes mellitus patients in Nigeria, and therefore, they could be at increased risk of cardiovascular disease.

**Keywords: Adropin, diabetes mellitus, dyslipidaemia, hyperglycaemia**