# Tuesday, August 1, 2017

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

Endocrinology/Hormones

## A-155

### Easy as 1, 2, 3? Trimester-specific TSH Reference Intervals in a Well-Characterized Population Using the Beckman Coulter 3rd IS Immunoassay

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**Background:** Lack of harmonization of thyroid stimulating hormone (TSH) immunoassays has led to variable reference intervals (RIs) and disagreement within the scientific community. This is concerning, as a clear understanding of what is "normal" is necessary to diagnose disease. Thyroid function changes throughout pregnancy make establishing RIs in this population additionally complex. Commercial immunoassays also use different reference standards, further confounding comparisons between studies.

**Objective:** The aim of the current study was to establish TSH RIs for Beckman Coulter immunoassays using well-characterized samples from pregnant and non-pregnant individuals. These assays utilize the more recent WHO 3rd international standard (IS).

**Method:** 1571 healthy U.S. subjects ≥18 years (y) were prospectively enrolled in an 8-center study. Subjects had no personal or family history of thyroid disease and were not using prescription medications. 164 subjects with thyroid peroxidase >9 IU/mL or thyroglobulin antibodies >4 IU/mL were excluded. RIs were established for women in their first (≤13 weeks), second (14-26 weeks) and third (≥ 27 weeks) trimesters of pregnancy, as well as men and non-pregnant women. Subjects included 318 women in first trimester pregnancy, mean age 27 y (SD 5.1), 84% Caucasian, 22% Hispanic, 16% Other; 362 second trimester, mean age 28 y (SD 5.6), 84% Caucasian, 32% Hispanic, 16% Other; 334 third trimester, mean age 27 y (SD 5.5), 84% Caucasian, 27% Hispanic, 16% Other; and 393 men and non-pregnant women (198 male, 195 female), mean age 41 y (SD 15.9), 77% Caucasian, 23% Hispanic, 23% Other. One third-trimester subject was excluded using Reed-Dixon rule. TSH was measured using the Access TSH (3rd IS) assay on Beckman Coulter Immunoassay Analyzers (UniCel DxI 800 and Access 2).

**Results:** The central 95th percentile RIs for the UniCel DxI 800 (Access 2) were determined to be 0.15-3.25 µIU/mL (0.15-3.33 µIU/mL) in first trimester pregnancy, 0.37-4.05 µIU/mL (0.37-4.20 µIU/mL) in second trimester, 0.48-4.41 µIU/mL (0.48-4.62 µIU/mL) in third trimester, and 0.50-3.92 µIU/mL (0.51-4.15 µIU/mL) for males and non-pregnant females. Upper and lower reference limits were contained within corresponding 95% confidence intervals between assays.

**Conclusion:** Similar to other methods and reports, TSH RIs increased as pregnancy progressed. While these trimester-specific upper RI limits were higher than some reports, there is considerable variation in the assays, standardization materials, populations, and statistics used in the literature. The male and non-pregnant female 95th percentile RI was consistent with previously published results using alternate methods, including those proposed in the 2002 NACB laboratory medicine practice guidelines. This highlights the importance of establishing RIs for individual assays and the critical need for TSH assay standardization.

## A-157

### Development of a New Biochip Based Immunoassay Unaffected by DHEA-S interference for the Accurate Measurement of Serum Progesterone

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**Background:** Accurate and reliable measurement of serum progesterone has important clinical implications as this hormone plays a significant physiological role in pregnancy**.** Progesterone levels are used by In Vitro Fertilisation (IVF) clinicians when deciding if implantation is feasible. A threshold, varying between 0.9-1.2ng/mL, is used to determine if the endometrium is receptive to implantation and if progesterone levels are found to be above this threshold fresh embryo transfer is postponed. Immunoassays used by many fertility clinics to assess the specific concentration of circulating progesterone are optimised for measurement of higher progesterone levels and lack the specificity and sensitivity required to identify small changes in these lower progesterone concentrations, which may impact patients undergoing IVF. The current study aimed at developing a new biochip based immunoassay for the specific measurement of progesterone at low concentrations in serum without interference with Dehydroepiandrosterone Sulphate (DHEA-S), which is prescribed for IVF preparation.

**Methods:** A direct competitive chemiluminescent immunoassay on a biochip platform with the fully automated Evidence Evolution was utilized. Assay sensitivity was determined as Limit of Blank (LOB), Limit of Detection (LOD) and functional sensitivity in accordance with Clinical and Laboratory Standards Institute (CLSI) guideline EP17-A2. Repeatability was determined following CLSI protocol EP05-A3: 2 runs per day in duplication for 20 days (n=80). A DHEA-S concentration of 20000ng/mL was used to determine interference, which was calculated as both cross reactivity and percentage interference in accordance with CLSI guidelines EP07-A2 and compared to two other CLIA systems. A correlation study was conducted by analyzing 44 serum samples and compared with an ECLIA assay.

**Results:** The analytical evaluation showed LOB, LOD and functional sensitivity values of 0.017ng/mL, 0.073 ng/mL and 0.122 ng/mL respectively. Repeatability was expressed as CV (%) for samples at the following concentrations; 1.130, 12.742, 46.020 ng/mL and was 5.5%, 5.5% and 6.9% respectively. When DHEA-S interference was evaluated, the biochip based assay showed -6.4% interference at 0.345 ng/mL of progesterone in comparison to other commercially available CLIAs, which showed 82.5% interference at 0.540 ng/mL of progesterone and 211% interference at 0.7 ng/mL of progesterone. In the correlation study, linear regression on the resulting data generated an r value of 0.981 for samples in the range of 0.39-53.5 ng/mL.

**Conclusion:** The results show that this new biochip based immunoassay for the determination of progesterone in serum, applied to the Evidence Evolution, a high throughput, random access with STAT capabilities, fully automated analyser, exhibits specificity, accuracy and precision for low concentrations. This device is a valuable and reliable analytical tool for the measurement of progesterone levels during IVF as it does not suffer interference from DHEA-S, which is frequently prescribed to patients preparing for IVF. Moreover as the biochip platform offers flexibility to incorporate multiple assays on the biochip surface, other steroids hormones can be simultaneously determined thus increasing the information to facilitate clinical understanding.

## A-158

### Autoimmune thyroid disease: Hashimoto s Thyroiditis is associated with low levels of Vitamin D in adults patients.

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Background. Autoimmune thyroid diseases (AITD) are common autoimmune disorders. Hashimoto's thyroiditis (HT) is one of the main clinical presentations of AITD and is characterized by lymphocytic infiltration of the thyroid parenchyma. The clinical hallmarks of HT is hypothyroidism, common findings are high serum concentration of thyroid stimulating hormone (TSH) and positive anti-thyroid peroxidase antibodies (ATPO). Evidence suggests that low levels of 25-hydroxy Vitamin D (Vitamin D) may contribute to the development of autoimmune disease; however, the relationship between Vitamin D deficiency and Hashimoto's thyroiditis is still controversial. The objective of this study is to investigate the association between serum TSH levels, positive ATPO and levels of Vitamin D in healthy and HT patients in the local population. Methods. The study was conducted on 190 patients drawn in our clinic between August and November 2016. The mean subject age was 56 ± 17 years old and the male/female ratio was 28 (14.7% male):162 (85.3% female). Pregnant women and patients with abnormal parathyroid hormone levels were excluded. All blood samples were collected in Spring to minimize the impact of seasonal fluctuations of Vitamin D concentrations. We measured TSH, FT4, ATPO and Vitamin D concentrations in healthy and hypothyroids patients. The cut off for positive ATPO was > 37 UI/mL, the normal reference intervals for TSH and FT4 were 0.40 to 4.00 µU/ml, and 1.00 to 1.80 ng/dl, respectively. Deficiency for Vitamin D was defined as serum concentrations below 30 ng/mL. TSH, FT4, ATPO and Vitamin D concentrations were determined using a chemiluminescent microparticle immunoassay (CMIA) on the Advia Centaur XP (Siemens, Germany). Data obtained for all measurements of Vitamin D was analyzed with Welch´s Test. TSH and FT4 in both groups was analyzed using the Mann Whitney U test. A p value < 0.05 represented a significant difference. Data was

expressed as mean ± error of the mean (SEM). Results. TSH serum concentrations were significantly increased in hypothyroid patients compared with control patients (4.22 ± 0.51 µU/ml vs 2.20 ± 0.11 µU/ml, $p < 0.05$). Patients with elevated ATPO had lower concentrations of Vitamin D than the control group (19.13 ± 0.68 ng/mL vs 22.61 ± 0.64 ng/mL, respectively, $p < 0.05$). FT4 concentrations showed no significant difference between hypothyroid group and control group (1.27± 0.19 vs 1.28 ± 0.11, $p < 0.05$). Conclusions. Results from the present study support the idea that Hashimoto's thyroiditis is associated with female gender, positive ATPO, high levels of TSH, and Vitamin D deficiency. We observed that serum Vitamin D concentration is significantly lower in HT patients in comparison to the control group. This suggests Vitamin D deficit may be one of the risk factors for HT development. Importantly, low levels of Vitamin D were observed in control group. We recommend supplementation with Vitamin D in general population.

## A-162

### COMPARATIVE STUDY OF LIVER ENZYMES IN UNCOMPLICATED TYPE 2 DIABETICS AND APPARENTLY HEALTHY INDIVIDUALS AT THE UNIVERSITY COLLEGE HOSPITAL IBADAN, NIGERIA

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**Background:** Type2 Diabetes mellitus is of major public health concern worldwide. Previous studies have shown that individuals with type 2 diabetes have higher incidence of liver function test abnormality than individuals without diabetes. There is however scarcity of information on liver enzymes in type 2 diabetics in our community. This study therefore investigated the plasma levels of AST, ALT and GGT in type 2 diabetics attending the endocrinology clinic at the University College Hospital, Ibadan, Nigeria.

**Methods:**The laboratory records of liver function tests of uncomplicated type 2 diabetics and apparently healthy individuals from January to November 2016 of our laboratory was compiled. The liver enzymes investigated were Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Gamma Glutamyl Transferase (GGT). Cobas C311 was used for the analysis of our assays. Levels 1 and 2 quality control material produced by Roche was always included in our daily work. The Reference Range of AST employed in our laboratory was 0- 37 IU/l, that of ALT was 0-40 IU/L while that of GGT was 7- 50 IU/L. IBM version 20 was employed for statistical analysis.

**Results:** Age range of type 2 diabetics of this study was 42-85 years with a mean of 63.32 ± 10.91 years while the age range of apparently healthy individuals was 40-89 years with a mean of 61.40 ± 11.82 years , $p = 0.378$. About 11.5% (7/61) of type 2 diabetics had elevated AST as compared to 6.7% apparently healthy individuals (4/60), $p = 0.529$. Also 4.9% (3/61) of type 2 diabetics had elevated ALT as compared to 5.0% (3/60) of apparently healthy individuals, $p= 1.000$. Moreover 35.6% (21/59) of type 2 diabetics had raised GGT compared to 27.1% (15/59) in apparently healthy individuals, $p= 0.428$. Mean value of AST in type 2 diabetics was 24.67 ± 12.41 while that of apparently healthy individuals was 24.08 ± 11.57, $p = 0.788$. On the other hand mean value of ALT in type 2 diabetics was 21.95 ± 19.63 and that of apparently healthy individuals was 18.80± 15.44, $p= 0.329$.Also the mean GGT levels in type 2 diabetics was 51.12 ± 32.73 and that of apparently healthy individuals was GGT 42.02 ± 24.01 , $p= 0.088$.

**Conclusion:**

Elevated AST, ALT and GGT do not seem to be a feature in patients on treatment for type 2 diabetics at the University College Hospital, Ibadan, Nigeria. Glycaemic control should be strongly advocated in diabetics patients in Ibadan, Nigeria.

## A-164

### Serum leptin level in Hypothalamic Amenorrhea

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**ABSTRACT Background** Researches support functional hypothalamic amenorrhea (FHA) is weight-loss, stress, and exercise-related condition which results from aberrations in pulsatile gonadotropin-releasing hormone (GnRH) secretion causing impairment of the gonadotropins (FSH/LH). The final consequences are complex hormonal changes manifested by profound hypoestrogenism. A sensitive marker of nutritional status, leptin is known to correlate with fat mass and to respond to changes in caloric intake. Leptin is an adipocyte-secreted hormone that plays a key part in energy homoeostasis. Studies in animals and human beings have shown that low concentrations of leptin are fully or partly responsible for starvation-induced changes in neuroendocrine axes, including low reproductive, thyroid, and insulin-like growth factor (IGF) hormones.**Objectives** This study aimed to determine serum leptin level in hypothalamic amenorrhea, to correlate serum leptin level with BMI and to compare serum leptin with other types of secondary amenorrhea viz; hyperprolactinemia, PCOS and hypothyroidism.**Methods** It is a single center, cross sectional, observational study. A total of 90 participants from gynecology OPD were enrolled in this study within 10 months. 81 cases were of secondary amenorrhea who had amenorrhea more than 3 months duration excluding pregnancy. They were divided into hypothalamic amenorrhea (42), hyperprolactenimia (19), hypothyroid (6), PCOS (14) and 9 eumenorrheic cases were age, height and weight matched with FHA. SPSS ver. 20.0 was used to analyze the data. T test and ANOVA were used to find mean differences and Pearson's correlation was used to establish the correlation between study variables.The p value less than 0.05 is considered significant. **Results** Mean age of study population was 25.3±5.2 years. Among all 38% of study population was of age group 20-25 years. Within the secondary amenorrhea group 47% were of hypothalamic amenorrhea followed by 21% hyperprolactinemia, 16% PCOS and 10% hypothyroidism. The weight and BMI of the hypothalamic amenorrhea cases were found to be significantly lower than other causes of amenorrhea ($p<0.001$). The mean serum leptin level was found to be lower in hypothalamic amenorrhea compared to other causes of amenorrhea and eumenorrheic control group (3.019±1.1 ng/ml vs. 9.315±4.2 ng/ml vs; 5.60±2.40 ng/ml, $p =0.001$). While in PCOS; BMI and serum leptin level were higher. Likewise serum TSH, LH, FSH, estrogen and testosterone were also found lower in hypothalamic amenorrhea compared to other types of amenorrhea ($p<0.001$). The cut off value of serum leptin in hypothalamic amenorrhea was found to be 4.45ng/ml from other causes of amenorrhea and control group. There were positive correlations between serum leptin and BMI, LH, FSH, TSH, estrogen and testosterone ($p<0.001$). **Conclusion** This study showed that serum leptin, weight and BMI level is significantly lower in hypothalamic amenorrhea than other types of amenorrhea and normal eumenorrheic control. The positive correlations between leptin and gonadotropins, estrogen, testosterone and TSH reflect the reproductive role of leptin in the HPG axis. Thus, leptin may act as the critical link between nutritional adequacy and the reproductive system, indicating whether adequate energy is present for normal reproductive function. **Key Words** Hypothalamic amenorrhea, Serum leptin, BMI, Nutrition, Gonadotropins

## A-165

### Thyroid-Related Testing Utilization: A Multi-Center Benchmark Study

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**Background:** Test utilization improvements require better knowledge of practice variation. Thyroid tests are some of the most commonly performed laboratory tests, yet little is known is known about the thyroid test ordering patterns. The objective of this study was to analyze practice variation in thyroid-related testing and to determine the impact of laboratory utilization management programs on testing patterns. **Methods:** 82 sites across the United States participated in the study. A survey was conducted to collect annual thyroid-related test volume data and utilization management activities. The thyroid-related tests examined included thyroid stimulating hormone (TSH), free thyroxine (FT4), total thyroxine (TT4), free triiodothyronine (FT3), total triiodothyronine (TT3), triiodothyronine uptake (T3U), and reverse triiodothyronine (rT3). Annual complete blood count (CBC) volumes were also collected to normalize TSH test volume (TSH/CBC), which served as a comparator for thyroid workup rates across sites. Individual thyroid testing volumes were normalized to that of TSH to compare thyroid test selection patterns. Quality of thyroid test ordering was assessed using the following test volume ratios: FT4 to T4-related tests (both FT4 and TT4) ratio, and T3U to TSH ratio. We also collected data on laboratory utilization management activities at each organization. **Results:** The thyroid workup rate (TSH/CBC) was higher for outpatient (0.26), relative to inpatients (0.03). Significant variation in test selection patterns were observed across sites for all tests. Based on the median values, 14 FT4, 3 TT4, 4 FT3, 2 TT3, 0.1 T3U, and 0.1 rT3 tests were ordered for every 100 TSH tests ordered. Approximately 90% of the T4-related orders were FT4 rather than TT4. T3-related orders (FT3 and TT3) were roughly evenly distributed between FT3 and TT3. While most of the organizations had implemented test utilization management activities to varying degrees, there was a weak relationship between the extent of these activities and the quality of thyroid test ordering. For instance, high quality thyroid test ordering would be suggested by a high FT4 to T4-related tests volume ratio, and a low T3U to TSH test volume ratio. FT4/T4 was positively correlated with utilization management activities ($r=0.38$) but the association was not statistically significant ($p = 0.15$). T3U/TSH had a statistically

significant negative correlation with utilization management activities (r = -0.54, p = 0.03). **Conclusion:** The test ordering patterns for analytes such as FT4 were consistent with guideline recommendations in the literature, e.g., the preferred use of FT4 over TT4 during workup. However, based on our sample, there still appears to be wide variation in thyroid-related test ordering patterns in the United States. As such, better implementation of more stringent test utilization management activities may be beneficial. Together, these results suggest that there remains much room for improvement in thyroid test utilization for a number of organizations, and that clearer guidelines may be warranted.

## A-166

### Development of a New Biochip Array Applied to the New Random Access Fully Automated Evidence Evolution Analyser for the Simultaneous Measurement of TSH, Free T4 and Free T3

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**Background**

Thyroid function tests are indicated in the diagnosis and management of thyroid disorders and most commonly Thyroid Stimulating Hormone (TSH), Free Thyroxine (T4) and Free Triiodothyronine (T3) are measured. TSH is secreted from the pituitary gland and it has been suggested to be the most sensitive indicator of hypo- or hyperthyroidism. TSH regulates thyroidal secretion of the thyroid hormones T4 and T3, which in turn exert a negative feedback on the pituitary and hypothalamus. A multi-analytical tool allowing the simultaneous measurement of these three hormones is therefore advantageous in clinical settings. This study reports the development of a new biochip array for the multiplex measurement of TSH, FT4 and FT3 from a single sample and applied to the first high throughput, random access with STAT capability, fully automated biochip analyser, Evidence Evolution. This application represents a new multi-analytical tool in the investigation of thyroid function.

**Methods**

Simultaneous chemiluminescent competitive and sandwich immunoassays were developed and applied to the biochip analyser Evidence Evolution, the capture antibodies being immobilised on the biochip surface at discrete test sites. Functional sensitivity was assessed along with repeatability precision using serum based precision material. Serum patient samples (n=53) were assessed and the results compared with commercially available methods.

**Results**

The biochip assay showed a functional sensitivity value of 0.01 µIU/mL for TSH. Repeatability assay precision values for low, medium and high levels of TSH, FT3 and FT4, expressed as CV (%) were 3 %, 3 % and 8 % CV for TSH, 4.7 % 3.8 % and 6.9% for FT3 and 2.9 % 2.6 % and 4.9% for FT4. R values of 0.99 for TSH, 0.98 for FT3 and 0.97 for FT4 were obtained following regression analysis of the results after the assessment of the 53 serum samples with the biochip assay and another commercially available methods.

**Conclusion**

The results show applicability of the newly developed biochip array for Evidence Evolution for the reliable simultaneous quantitative determination of high sensitive TSH, alongside FT3 and FT4 from a single serum sample. This multi-analytical approach will aid in the efficient diagnosis and management of patients with thyroid disorder. The new Evidence Evolution platform also incorporates STAT sample and random access capabilities.

## A-167

### Method-Specific Reference Intervals for Thyroid Function Tests during the Third Trimester of Pregnancy

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**Background:** The American Thyroid Association recommends trimester- and method-specific reference intervals (RI) for markers of thyroid function during pregnancy. Study objectives were to establish RIs for thyroid stimulating hormone (TSH) and free thyroxine (FT4) during the 3rd trimester of pregnancy using the Roche cobas e602. This expands upon our previously reported RIs for 1st and 2nd trimesters.[1,2]

**Methods:** Surplus maternal serum screen specimens were collected from 157 subjects ranging from 15-43 years of age (median=26 years), with gestational age of 27-40 weeks (median=28.3 weeks). TSH and FT4 testing were performed using the Roche cobas e602. Thyroglobulin (TgAb) and thyroid peroxidase (TPOAb) autoantibodies were measured using the Beckman Coulter DxI. TgAb and/or TPOAb positive subjects were excluded from analyses (>4.0 and >9.0 IU/mL, respectively). The central 95% nonparametric RI for TSH was determined, and then FT4 RIs were determined using subjects within this TSH RI. Results were compared to previously determined RIs using self-reported healthy, non-pregnant subjects, and data from 1st and 2nd trimesters.[1,2] The RI for pregnant subjects was considered significantly different if the reference limits did not fall within the 90% confidence intervals (CI) of comparison group.

**Results:** TSH and FT4 RIs are summarized (Table). When comparing RIs from 3rd trimester subjects to non-pregnant subjects, the lower reference limit for TSH was not found to be significantly different; whereas the upper reference limit was significantly lower (*). For FT4, both the lower and upper reference limits for 3rd trimester subjects were significantly lower than non-pregnant individuals. Additionally, significant differences were observed between the three trimesters.

**Conclusions:** Significant differences for TSH and FT4 RIs were observed between the 3rd trimester of pregnancy and non-pregnant individuals, and between the three trimesters. This supports guidelines recommending trimester- and method-specific RIs for thyroid function tests.

[1]Silvio et al. ClinBiochem 2009

[2]Wyness et al. ClinChimActa 2011

| Analyte/ Population | n | 2.5th percentile (lower limit) | 90% CI (2.5th) | 50th percentile (median) | 97.5th percentile (upper limit) | 90% CI (97.5th) |
|---|---|---|---|---|---|---|
| **TSH (mU/L)** | | | | | | |
| 3rd trimester | 145 | 0.39 | 0.16 – 0.52 | 1.48 | 3.84* | 2.84 – 5.47 |
| Non-pregnant | 134 | 0.36 | 0.01 – 0.72 | 1.94 | 4.77 | 4.14 – 5.29 |
| **FT4 (ng/dL)** | | | | | | |
| 3rd trimester | 139 | 0.70* | 0.64 – 0.75 | 0.96 | 1.22* | 1.17 – 1.31 |
| Non-pregnant | 128 | 0.89 | 0.80 – 0.93 | 1.22 | 1.58 | 1.50 – 1.65 |

## A-169

### STATUS OF VITAMIN-D IN RELATION TO GLYCEMIC INDICES > LIPID PROFILE IN POST-MENOPAUSAL WOMEN WITH TYPE 2 DIABETES MELLITUS

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**Background:** Abnormal vitamin D level and glucose homeostasis are two of the most chronic medical conditions leading to osteoporosis and cardiovascular disease following menopause transition in females. Vitamin D deficiency is the most commonest health problem among postmenopausal women worldwide. Low levels of vitamin-D could be associated with elevated risk of cardio metabolic disorders comprising cardiovascular disease and type 2 diabetes. Besides enduring multiple complications of chronic hyperglycaemia, diabetic patients tend to be soft targets of deadly cardiovascular disease (CVD) due to dyslipidemia. The aim of the present study was to evaluate and compare vitamin D status in relation to glycemic indices ,lipid profile between premenopausal and postmenopausal women with type 2 diabetes (T2DM).

**Methods:** : In this cross sectional study, 600 women with T2DM were divided in pre-menopausal (n = 300) and post-menopausal (n = 300) group. Levels of fasting blood glucose , HbA1C, lipid profile parameters, i.e., total cholesterol (TC), triglycerides (Tg), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and vitamin D were measured in pre and postmenopause women and analysed by SPSS software. Comparison between the groups was done by one way ANOVA followed by Holm-Sidak test .

**Results:** The mean ages of premenopausal and postmenopausal were 43.16 ± 4.2 and 59.59±10.08 years, respectively. Levels of HbA1C, FBG, TC, Tg and LDL-C increased significantly ($p<0.001$) in postmenopause women compared to premenopausal women. In contrast to these parameters, serum levels of HDL-C, Vitamin D decreased significantly in T2DM postmenopause diabetic women compared to premenopausal diabetic women. Vitamin-D was negatively correlated with age,HbA1C,LDL-C at $p<0.05$. This study had shown that dyslipidemia in postmenopausal diabetic women had higher prevalence of high Tg, TC, and LDL-C than the pre-menopausal women, indicating that they were more prone to cardiovascular diseases.

**Conclusion:** Dyslipidemia observed in postmenopause women accompanied with decreased vitamin-D increases the risk factors in Type 2 Diabetes.

## A-170

### Validation of optimized saliva immunoassays for Testosterone, Progesterone and Cortisol.

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Between 95 and 99% of a hormone in the bloodstream is bound to carrier proteins, and only the unbound fraction freely diffuses into tissues, including the salivary gland. Therefore, saliva is a clinically informative, biological fluid that is useful for novel approaches to prognosis, laboratory or clinical diagnosis, and monitoring and management of patients with both oral and systemic diseases. It is easily collected and stored and ideal for early detection of soluble biomarkers, because both diurnal and monthly profiles of hormone levels parallel traditional serum patterns. Here we present validation data that confirm that the analytes testosterone, progesterone and cortisol can be measured with good precision and sensitivity from oral fluid. Furthermore, results perfectly correlate to mass spectrometry results. All assays have a total assay time of 1.5 hours, and need 100 µl of saliva sample. Spiking recovery and linearity were proven to be in the range of 100 +/- 15%. Salivary Testosterone (SLV-3013): Measurement of testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones, including primary and secondary hypogonadism, delayed or precocious puberty, impotence in males and, in females hirsutism, and virilization due to tumors, polycystic ovaries, and adrenogenital syndromes. Assay characteristics are: Measuring range: 2.63 (LoD) - 1000 pg/ml. LoQ: 10.1 pg/ml. Mean intra-assay precision: 4.7%, mean inter-assay precision: 7.6%. Method comparison showed very good correlation to LC-MS/MS (r = 0.9904; y = 1.015 x - 2.8203) and normal ranges were determined for men (age-dependent) and women. Salivary Progesterone (SLV-5911): The steroid hormone Progesterone is a female sex hormone which, in conjunction with estrogens, regulates the accessory organs during the menstrual cycle and it is particularly important in preparing the endometrium for the implantation of the blastocyte and in maintaining pregnancy. Assay characteristics are: Measuring range: 1.1 - 2400 pg/mL. Mean intra-assay precision: 6.3%, mean inter-assay precision: 9.2%. Method comparison showed very good correlation to LC-MS/MS (r=0.997; y = 0.9612x - 11.071) and normal ranges were determined for women in follicular and luteal cycle phase as well as men. Salivary Cortisol (SLV-2930): Cortisol shows a diurnal rhythm with highest concentrations in the morning and steady decrease to very low levels 12 hours later. Cortisol secretion increases in response to any stress in the body, whether physical (such as illness, trauma, surgery, or temperature extremes) or psychological. Moreover, elevated cortisol levels and lack of diurnal variation have been identified with Cushing's disease and in patients with adrenal tumors. Low cortisol levels are found in primary adrenal insufficiency (e.g. adrenal hypoplasia, Addison's disease) and in ACTH deficiency. Assay characteristics are: Measuring range: 0.09 - 30 ng/mL. Mean intra-assay precision: 3.9%, mean inter-assay precision: 7.4%. Method comparison showed very good correlation to LC-MS/MS (r=0.999; y = 1.032x +0.111) and normal ranges were determined for men and women at morning, noon and evening.

## A-171

### Mean platelet volume and diabetes in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil)

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**Background:** Diabetes Mellitus (DM) is associated with higher risk of atherothrombosis, and 80% of patients with DM died because of thrombosis whose principal trigger is endothelial dysfunction and platelet hyperactivity. Under physiological conditions, the number of platelets is inversely proportional to mean platelet volume (MPV), to keep a constant level of platelet mass. Studies in DM patients show that the balance between platelet production and depletion is lost, and they tend to have higher MPV values without difference in the platelet count. Our purpose in this study was to investigate whether diabetes and pre- diabetes are independently associated with MPV, an easily obtained marker of platelet size and platelet activity. **Methods:** We used the baseline data (2008-2010) of 3115 civil servants (aged 35-74 yr) from a university and enrolled in the Brazilian Longitudinal Study of Adult Health (ELSA-Brasil). Venous blood sampling was performed after 12- to 14- hour-fasting, using tubes containing ethylenediaminetetraacetic acid (EDTA). The time between sampling and exam procedure was strictly controlled to be within 2 hours, and blood samples were kept at room temperature until the measurements. Presence of DM were classified using fasting plasma glucose (FPG; ≥126 mg/dL [7.0 mmol/L]), 2-hour plasma glucose (PG) during an oral glucose tolerance test (OGTT) (2h PG OGTT; ≥200 mg/dL [11.1 mmol/L]), and glycated hemoglobin (HbA1c; ≥6.5%; [48.0 mmol/mol]). DM was also defined by the self-reported information or use of insulin or hypoglycemic medication identified in the baseline survey of the ELSA study. Pre-diabetes was classified by the presence of impaired fasting glucose (IFG) (FPG ≥ 100 mg/dL [5.6 mmol/L] to 125 mg/dL [6.9 mmol/L]), and/or impaired glucose tolerance (IGT) (2h PG OGTT ≥140 mg/dL [7.8 mmol/L] to 199 mg/dL [11.0 mmol/L]), and/or HbA1c ≥5.7% (39 mmol/mol) to 6.4% (46 mmol/mol), according to ADA. Multiple linear regression analysis was used to estimate the independent association of the diabetes and pre-diabetes with the MPV after adjusting for sex, age, platelet count, and hypertension. All the variables entered in the multiple regression analysis using the forward approach. Statistical assumptions to perform multiple linear regressions were checked by residual analysis. **Results:** MPV (adjusted $r^2$=0.143; p=0.01), was independently associated with diabetes and pre-diabetes, compared to normoglycemic subjects. The set of variables included in the multivariate model remained explained about 14% of the variability of the MPV evaluated. Diabetes had higher β (0.207) that than pre-diabetes (β=0.110) in the model to estimate the independent association with MPV. **Conclusion:** In this large cohort of free living Brazilians, ours results showed that increased MPV is independently associated with the presence of diabetes and pre-diabetes, suggesting an early change in initial increase of the glucose levels. Platelets from diabetic patients are an accelerated rate of renewal, so higher MPV values may act as a marker of the production of bigger, denser, and more reactive platelets in DM type 2. Whether this condition is the cause or consequence of atherothrombotic cardiovascular events in diabetics remains unclear.

## A-172

### Biomarker changes in adult men with low testosterone (low-T)

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**Background:** Androgens such as testosterone are known to have effects on many organs and systems, such as prostate, bone marrow, bone turnover, muscle, and metabolism. However, it is not known if men with androgen deficiency (low-T) have consistent or characteristic abnormalities in the biomarkers that measure the functions of the organs and systems that are influenced by androgens. The purpose of this retrospective study was to compare the mean levels of various biomarkers in men with low-T (n=1752) and in men with normal T (n=9617).

**Methods:** The Utrecht Patient Oriented Database (UPOD) contains all health care data and measurements from all patients admitted to the University Medical Center Utrecht in the Netherlands. We extracted data from male patients over 40 years old who presented for evaluation of possible low-T and who had a laboratory measurement of total testosterone levels in combination with a measurement of one or more of the following biomarkers on the same day: free testosterone (n=6264), uric acid (n=308), estradiol (n=1016), prostate specific antigen (PSA, n=2897), sex-hormone binding globulin (SHBG, n=7126), luteinizing hormone (LH, n=4422)), creatinine (n=6781), bone alkaline phosphatase (BAP, n=3421), creatine kinase (n=167), LDH (n=2829), hemoglobin A1c (n=2249), and 25-hydroxy-vitamin D (n=856). Measurements from patients having a diagnosis of prostate cancer were excluded. Analyses were stratified based on serum testosterone levels classified into lowest (<4.5), low (4.5-7), and normal (>= 7 mmol/L). Differences between testosterone strata were assessed with the Kruskal Wallis test.

**Results:** Compared to men with normal levels of T, the men with the lowest levels of T had significantly (p<0.001) lower means of free testosterone (51 versus 300 pmol/L); PSA (0.49 versus 0.94 micrograms/L); SHBG (29 versus 35 nmol/L); luteinizing hormone (1.5 versus 3.6 IU/L); and estradiol (40 versus 89 pmol/L). In comparison to men with normal levels of T, men with low levels of T also had statistically (p<0.001) higher mean levels of LDH (217 versus 198 U/L); BAP (81 versus 75 U/L); and hemoglobin A1c (41 versus 39 mmol/mol). Mean uric acid levels in men with the lowest T levels were also higher than in men with normal T (0.41 versus 0.34 mmol/L, p=0.02).

**Conclusion:** Our results indicate that low T in adult men is associated with significant changes in various biomarkers that measure the functions of organs and systems that are influenced by androgens, such as prostate, bone, and the endocrine system. This finding is important because it may lead to improved diagnosis and treatment of low-T by identifying those men who have objective evidence of physiologic changes produced by androgen deficiency that may warrant therapy.

## A-174

### The Study of Trimester-Specific Thyroid Stimulating Hormone and Free Thyroxine Reference Intervals with Chinese Women by Experimental and Statistical Methods

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**Background:** As a result of physiological and metabolic changes during pregnancy, thyroid hormones can be affected significantly throughout entire three trimesters. For example, two pregnancy-related hormones—human chorionic gonadotropin (hCG) and estrogen, are well known to cause increased thyroid hormone levels in the blood. To support thyroid disease diagnosis in pregnancy, the objective of this study was to establish trimester-specific thyroid stimulating hormone (TSH) and free thyroxine (FT4) reference intervals (RIs) in Chinese women by experimental and statistical methods.

**Methods:** A total of 1205 pregnant women were recruited from Jan 2016 to Dec 2016 at our hospital according to the following exclusion criteria: Patients who are with a personal or family history of thyroid disease, with a goiter, have more than one fetus, or pregnancy complications. Those initially selected patients were further tested for TSH, FT4 and thyroid peroxidase antibody (aTPO), performed on the chemiluminescent platform Siemens ADVIA Centaur® XP. Only patients tested negative for aTPO were included in reference interval establishment. Besides, linear regression was carried out between FT4 and log transformed TSH to see if there is a linear correlation. Lastly, to validate the Hoffmann indirect method for the derivation of TSH and FT4 RIs, 10044 outpatients who came to our institute in 2016 for thyroid function screening in their first trimester (1-13 week) were included. The reference change value (RCV) was calculated for determining the statistical significance of the differences between the calculated RIs by Hoffmann method and the observed RIs in this study.

**Results:** According to the CLSI recommendation, RIs for both TSH and FT4 were determined as 2.5$^{th}$ percentile to 97.5$^{th}$ percentile on the data distribution. The TSH and FT4 trimester-specific RIs were shown as follows: 0.59-3.56 mIU/L, 11.8-18.4 pmol/L (n=188, 1st trimester); 0.79-4.60 mIU/L, 11.6-17.5 pmol/L (n=133, 2nd trimester); 0.65-4.20 mIU/L, 9.6-15.1 pmol/L (n=157, 3rd trimester).. When compared pairwise with Mann-Whitney test, both TSH and FT4 levels were statistically significant between 1st and 2nd, 1st and 3rd, 2nd and 3rd (not for TSH). The RIs of TSH and FT4 determined by Hoffmann method for first trimester outpatient pregnant women were 0.33-3.96 mIU/L and 11.7-17.5 pmol/L respectively. There is no significant difference between observed and calculated RIs for first trimester pregnant women in this study. No linear relationship was observed between FT4 and logTSH in any trimester-specific population.

**Conclusion:** We have established trimester specific RIs for thyroid function test in a Chinese population using both experimental and statistical methods. The results of the two methods are comparable. The similar approach can be applied to evaluate and verify the trimester specific RIs for other analytes.

## A-175

### Performance Evaluation of the ADVIA Centaur Androstenedione Assay

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**Background:** Androstenedione is a 19-carbon steroid that serves as a precursor for testosterone and estrone. It is most commonly used in conjunction with other steroid assays to evaluate the function of the adrenal glands and ovaries or testes and to determine the cause of symptoms of androgen excess.

A new ADVIA Centaur® Androstenedione (ANDRO) assay for the measurement of androstenedione in human serum and plasma is being developed by Siemens Healthineers. The studies below describe preliminary performance of the assay on the ADVIA Centaur® Immunoassay System.

**Methods:** The ADVIA Centaur ANDRO assay is a fully automated competitive immunoassay using direct chemiluminescent technology. Reagents include a biotinylated sheep monoclonal antibody coupled to streptavidin-coated paramagnetic particles in the solid phase and a newly developed acridinium ester in the Lite reagent. The assay requires 20 µL of patient sample or calibrator, which is incubated with solid phase and Lite reagent. Competition for solid phase binding occurs between androstenedione in the sample and the Lite reagent. Separation follows, and the amount of signal generated is inversely proportional to the concentration of androstenedione in the sample. The time to first result is 18 minutes.

**Results:** LoQ studies and linearity evaluation of the ADVIA Centaur ANDRO assay demonstrated an assay range of 0.30 to 10.00 ng/mL; with automated dilution, the measuring interval was extended to 50.00 ng/mL. The assay correlated well with LC-MS/MS, and equivalent performance was obtained using serum, lithium heparin, and EDTA plasma tube types. The assay showed ≤10% interference for all interferents tested and ≤1% cross-reactivity for all endogenous and most exogenous cross-reactants evaluated. Within-lab precision was <9% CV (with 95% confidence) across the assay range. Stability data demonstrated a calibration interval and onboard stability of 20 days and 16 days, respectively.

**Conclusions:** The ADVIA Centaur ANDRO assay demonstrates good precision and correlates well to LC-MS/MS.

*Information about this device is preliminary. Safety and effectiveness for the uses discussed have not been established. The device is under development and not commercially available. Future availability cannot be ensured.

## A-176

### Critical values in the endocrinology laboratory;our experience

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**Background:** Critical Values(CV) are results of diagnostic tests that express a medical situation wich may put the patient's life at risk if nothing is done properly and on time. Many clinical situations in Endocrinology could generate results of CV in laboratory parameters. According to available literature and in conjunction with specialized professionals of our staff we defined the following Endocrine disorders that could compromise patients life: Myxedematous Coma,Thyroid Storm, Acute Adrenal Crisis, Acute Abdomen in Assisted Fertilization and Trophoblastic disease.

Once the professional staff of the laboratory chooses to determine a program of CV, they must clearly define a policy which should include the list of tests, the mechanisms and people responsible for notifying the CV when they occur.The frequency of the CV, is highly variable and depends on the type of population served and other characteristics of each institution.OBJECTIVE: To evaluate the frequency of CV in our laboratory along a year after we defined policy of them regardless of whether they are inpatients or outpatients. Also report the time of clinical evolution in the Electronic Health Records (EHR).

**Methods:** In order to develope a documented system for CV we define the following list of serum determinations: Thyrotropin(TSH)>100.0uUI/mLTotalThyroxine(T4)>20.0ug/mL; Free T4<0.4 and >4.0ng/dL; Estradiol(E2)>4000pg/ml; BHCG>500000mU/mL performed in Architect i2000(Abbott) and Cortisol at 8 pm without corticosteroids:<5 ug/dL in Immulite 2000(Siemmens) and we determine the frequency of them. Both fully automated and *chemiluminescent* analyzers. The records in the EHR were divided into four groups according to the time of delay in the evolution differentiating between inpatients outpatients.

**Results:** Total number per year (TN/Y) of Cortisol is n=2619, number of CV per year (CVn)=93 (3.6%); E2 TN/Y=7029 CVn=15(0.2%); BHCG TN/Y=4697 CVn=2(0.04%);TSH TN/Y=95013 CVn=58(0.06%);T4L TN/Y=31920 CVn=20(0.06%);TotalT4 TN/Y=23286 CVn=15(0.06%).The frequency of the total CVs per year is: Cortisol (41%) followed by TSH(37%), T4(7%), E2(7%), T4L(6%), T3(1%) y BHCG(1%). The percentage of clinical evolutions in the EHR within the first hour of recording the CV (inpatients/outpatients)(30.1%/9.8%); between 1 and 6 hours(21%/5.6%); between 6 and 24 hours (7.7%/0.7%) and more than 24 hours (18.2%/1.4%).

**Conclusion:**Cortisol was the most frequent parameter we found and it should be the first to be include in the list.TSH despite being the most requested determination in our laboratory was not the most common CV probably due to extensive knowledge of this pathology.

The policy of CV, rather than a rule or a tool for continuous improvement in the clinical laboratory is a right for patients and the circuit is closed when the doctor records and takes corrective action.The CV reporting process is an important laboratory resource to maximize clinical benefits. Due to insufficient information of CV in the Laboratory of Endocrinology our intention is to provide our experience to improve the quality of them.

## A-177

### The functional SNP and expression of IL15 gene are associated with the development of autoimmune thyroid disease.

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[**Background**]There are considerable differences in the prognosis of autoimmune thyroid diseases (AITDs) including Graves′ disease (GD) and Hashimoto′s disease (HD). It has been known that the genetic producibilities of some cytokines and immune modulators are associated with their prognosis.

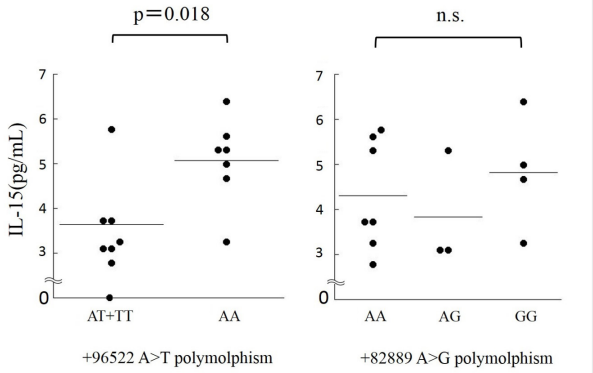
IL-15 is a proinflammatory cytokine and produced by several cells such as monocytes and activated CD4+ T cells. In various autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and HD, higher serum levels of IL-15 have been reported, suggesting that IL-15 may be associated with the onset of autoimmune diseases.

**[Methods]**To clarify the association between the genetic producibility of IL-15 and the pathogenesis of AITDs, we genotyped +96522 A>T and +82889 A>G polymorphisms in the *IL15* gene using 127 patients with HD, including 55 patients with severe HD and 48 patients with mild HD; 130 patients with GD, including 52 patients with intractable GD and 44 patients with GD in remission; and 79 healthy volunteers.

**[Results]** Both the *IL15* +96522 A allele and AA genotype were more frequent in patients with severe HD than in those with mild HD. The serum levels of IL-15 were higher in individuals with the *IL15* +96522 AA genotype than in those with the T allele, and they were also higher in patients with severe HD than in those with mild HD. On the other hand, the mRNA levels of IL-15 were not significantly different among individuals with each genotype of both SNPs. After incubation with recombinant human IL-15, the proportions of Th17 cells in $CD4^+$ cells were increased, and those of Treg cells in $CD4^+$ cells were maintained.

**[Conclusion]**Our study indicates that the *IL15* +96522A>C polymorphism correlates with the severity of HD, most likely by increasing Th17 cells.

The relation between polymorphisms of *IL15* gene and the pathogenesis of AITD.




## A-178

### Evaluation of the analytical performance of Tosoh G11 for $HbA_{1c}$ determination

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Background: Glycated hemoglobin($HbA_{1c}$) is a key biomarker for the monitoring of glycemic balance in diabetic patients. It can be measured by various methods, including ion-exchange high-pressure liquid chromatography (HPLC), boronate affinity chromatography, immunoassay method and capillary electrophoresis. The aim of this study is to evaluate the performance of new system, Tosoh G11 ( ion-exchange HPLC) in comparison to two other used system (Tosoh G8 and Biorad D-100) in routine testing.

Methods: 40 samples of whole blood in Anam Korea University hospital were collected from during January 2017. We evaluated analytical performance of new device, Tosoh G11. Within-run precision test was determined by 20 assays from same sample(quality control samples with different $HbA_{1c}$ values: two different levels, high and low, each sample being analyzed 20 times) on the same day. Between-day precision test was determined by daily measurement of $HbA_{1c}$ during 5 days, using two different quality control samples. The correlation with two other systems (Tosoh G8, biorad-D-100)was assessed by analyzing 40 samples. A test for linearity was investigated by preparing six different samples. Carry-over test was done by 4 high and low value samples each. Reference range analysis was done by CLSI C28-A3 that less than 10% of more than 20 samples must be in reference range provided by instructor.

Results: In within-run precision test, mean and coefficients of variation (CVs) for low and high value samples were 4.87%, 9.73%, and 0.97%, 0.57% respectively. In between-day precision test, CVs were less than 0.68%. The comparison of $HbA_{1c}$ values obtained using Tosoh G11 and Tosoh G8 showed a good correlation, with the following equation for the linear regression line: $y = 0.9664x + 0.2463$, and a coefficient of correlation, $R^2 = 0.9982$. In addition, Tosoh G11 and Biorad D-100 also showed a good correlation, with following equation for the linear regression line: $y = 1.0335x - 0.1587$, $R^2 = 0.9941$. New device exhibited a good linearity for $HbA_{1c}$ values ranging from 3.4% to 18.8%. The equation of the linear regression line was $y = 0.9762x + 0.0136$ with a correlation coefficient, $R^2 = 0.9999$. Result of carry-over test was 0.00%, less than 1%. In reference range analysis, none of the 20 samples was rejected.

Conclusion: In conclusion, this new device, Tosoh G11 showed good analytical performance at high throughput. Thus, the results of this evaluation suggest that the Tosoh G11 is suitable for a routine use in clinical chemistry laboratories.

## A-179

### Evaluation of the Beckman Access Free T3 Assay reference interval following an assay formulation change

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**Background:** Free triiodothyronine (fT3) is a second- or third-line test in the evaluation of hyperthyroidism. In our laboratory, fT3 measurements are performed using the Beckman Access Free T3 assay on the Unicel DxI platform. On March 2016, following the implementation of a new reagent lot, an increase in the frequency of elevated fT3 results was observed despite an acceptable lot evaluation. The average historical frequency of abnormal results was 16% using a reference interval of 2.0-3.5 pg/mL and increased to 40% with the new fT3 lot. Following discussions with manufacturer, it was concluded that the new reagent lot contained a different formulation design than our prior lots. This formulation change was introduced to improve the Access Free T3 reagent pack stability and resulted in the upward shift in fT3 concentrations. Although the manufacturer did not update the reference interval, the medical device recall letter indicated that laboratories should discontinue the use of the assay until the reference intervals were verified, adjusted or reestablished by the laboratory. The goal of this study was to establish the fT3 reference interval with the new fT3 reagent formulation.

**Methods:** Free T3 concentrations in serum from 129 individuals (71 (55%) male, 58 (45%) female) were determined. The participants were excluded if they have the following conditions: any thyroid disease, endocrine disorders, kidney disease or failure, liver disease, pregnancy, high iodine diet or hospitalization within the last 3 months. The following medications were also excluded: thyroid medications, amiodarone, lithium, glucocorticoids, propranolol, phenytoin, carbamazepine, furosemide, and hormone replacement (estrogen, testosterone). Samples were tested for thyroid stimulating hormone (TSH), Free T4, thyroperoxidase antibody (TPO), and thyroglobulin antibody to assure normal thyroid status. The central 95th percentile reference interval and the confidence intervals were calculated using quantile regression methods (SAS QUANTREG).

**Results:** Verification of the manufacturer's reference interval of 2.1-3.9 pg/mL with a small sample was unacceptable with only 80% (23/29) of results within the reference interval. A new reference interval was established with 129 individuals. The calculated central 95th percentile reference interval was 2.8-4.4 pg/mL. The $95^{th}$ confidence intervals were 2.7-2.9 and 3.9-5.0 for the $2.5^{th}$ and $97.5^{th}$ percentiles, respectively. Retrospective evaluation of the new reference interval using fT3 results (n=4362) obtained with the new reagent lot showed a decrease of abnormal results from 40% to 20% which was in alignment with the historical frequency.

**Conclusions:** We were unable to verify the manufacturer's reference intervals using the new fT3 assay formulation. We established a new reference interval for the Beckman Access Free T3 assay to account for the upward shift in fT3 concentrations observed with the formulation. With the implementation of this reference interval the frequency of abnormal results decreased to match historical frequencies.

## A-180

### Free Thyroxine Concentrations in the Hypothyroidism Treated Versus Untreated Populations

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**Background:**

Thyroid stimulating hormone (TSH) and free thyroxine (FT4) are integral tests for assessing thyroid function and guiding therapy. Although a normal TSH is the primary endpoint for patients being treated for hypothyroidism, FT4 is often also measured. Unfortunately, reference intervals for FT4 are not applicable to treated patients and abnormal results create confusion both for clinicians and patients. Although it is known that FT4 is generally higher in treated patients than their untreated counterparts, few studies detail the extent of these elevations and none on a large scale.

**Objectives:**

To assess how FT4 concentrations differ in patients being treated for hypothyroidism versus those who are not.

**Methods:**

Paired TSH and FT4 results between February 16th, 2016 and September 26th, 2016 were extracted from the electronic medical record. Additional data included age, gender, thyroid medications, pregnancy status, and all other thyroid related tests including free triidothyronine (FT3), total thyroxine (TT4), total triiodothyronine (TT3), anti-thyroid peroxidase antibody (ATPO), anti-thyroglobulin antibody (ATG), thyroid stimulating immunoglobulin (TSI), and free thyroxine by dialysis (FT4D). With the exception of FT4D and TSI, all testing was performed on the Roche Cobas 8000. Unfiltered data included 24,297 unique clinical encounters for 19,898 patients. All data analyses and figures were created with R Statistical Package Version 3.3.1 and R Studio Version 0.99.902.

**Results:**

Two populations were designed to compare how patients with a normal TSH (0.30-5.60 uIU/mL) differ biochemically depending on whether or not they are receiving medications for hypothyroidism. The reference population (P1) includes patients that are not pregnant, have no detectable thyroid related autoantibodies, and are not being treated for hyper- or hypothyroidism (8,179 encounters of 7,972 patients). The treated population (P2) includes patients that are not pregnant and have a current prescription for a hypothyroidism treatment (5,985 encounters of 5,113 patients). For P1, FT4 (ng/dL) had a mean (μ) of 1.17, a median (M) of 1.16, and a standard deviation (s) of 0.19. For P2, FT4 had a μ=1.39, M=1.40, and s=0.27. The central 95th percentile for both populations was calculated parametrically and non-parametrically and gave similar results with 0.79-1.55 for P1 and 0.86-1.94 for P2. FT3 (pg/mL) was also measured in a subset of these patients. Here the reference population (692 encounters of 667 patients) had a μ=2.86, M=2.90, and s=0.45 and the treated population (613 encounters of 547 patients) had a μ=2.69, M=2.6, and s=0.62. FT4 in this subset maintained the relationship seen in the original populations.

**Conclusion:**

FT4 concentrations in patients being treated for hypothyroidism are shifted higher relative to their untreated counterparts. This shift is not accompanied by a corresponding shift in FT3. Although TSH is the primary guide for therapy, this data provides information to clinicians as to when a FT4 above the reference interval in a treated patient may warrant further investigation and testing.

## A-182

### Quantitative Determination of Thyroid Stimulating Hormone (TSH) in Human Serum by Lumipulse® *G* TSH-III Assay

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**Background:** TSH (thyroid-stimulating hormone) is a pituitary hormone that acts on the thyroid gland to produce and release thyroxine (T4), and triiodothyronine (T3); the hormones that stimulate metabolism. TSH concentrations in blood closely reflect changes in thyroid function and are routinely used for evaluation of patients suspected of having an excess (hyperthyroidism) or deficiency (hypothyroidism) of thyroid hormones.

**Methods:** The Lumipulse G TSH-III is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative measurement of TSH in specimens on the LUMIPULSE G1200 System by a two-step sandwich immunoassay method. TSH specifically binds to an anti-human TSH monoclonal antibody (mouse) coated on particles and forms immunocomplexes. After washing, an Alkaline phosphatase (ALP)-labeled anti-human TSH monoclonal antibody specifically binds to the TSH immunocomplexes, completing the sandwich. The amount of TSH is derived from the luminescence signals generated by adding the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt). The calibrators for the Lumipulse G TSH-III assay are traceable to in-house reference calibrators whose values have been assigned to the 3rd International Standard, 2003 (code: 81/565) by the National Institute for Biological Standards and Control (NIBSC). All of the validation studies were performed according to respective CLSI guidelines.

**Results:** The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)/Functional Sensitivity (FS) of the Lumipulse G TSH-III assay on the LUMIPULSE G1200 System were 0.001, 0.002 and 0.006 μIU/mL, respectively. The Lumipulse G TSH-III assay demonstrated linearity in the range from 0.001 to 227.804 μIU/mL. There was no high-dose hook effect observed for samples containing up to ~3,100 μIU/mL of TSH. A twenty day precision study of 6 human serum-based panels assayed in duplicate at two separate times of the day (n = 80 for each sample) demonstrated within-laboratory (total) precision of ≤ 6.4%. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 9 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum albumin, immunoglobulin G, biotin, human anti-mouse antibody, and rheumatoid factor) and 17 commonly used therapeutic drugs. Cross-reactivity of the Lumipulse G TSH-III assay with other substances (5000 mIU/mL FSH, 200,000 mIU/mL hCG, 100 ng/mL hGH and 1000 mIU/mL LH, respectively) that are similar in structure to TSH demonstrated no cross-reactivity. A comparison of Lumipulse G TSH-III with an FDA-cleared predicate device was analyzed using weighted Deming regression. For the 141 tested specimens (Concentrations range from 0.026 to 84.299 μIU/mL), the slope, y-intercept, and correlation coefficient (r) were 0.97, -1.051 μIU/mL, and 0.9838, respectively. Finally, reference intervals as defined by 2.5th and 97.5th percentiles of the population were established for Lumipulse G TSH-III in 119 euthyroid adults (0.392-3.762 μIU/mL); 89 hyperthyroid adults (0.021-2.086 μIU/mL) and 110 hypothyroid adults (0.036-47.725 μIU/mL).

**Conclusion:** The data demonstrate that the Lumipulse G TSH-III assay on the automated LUMIPULSE G1200 System is sensitive, accurate and precise for routine quantitative determination of TSH in serum specimens.

## A-183

### Accuracy-based proficiency testing for testosterone measurement - a follow-up study 2016

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**Background:** Accurate measurement of testosterone is important in patient care and public health. Although proficiency testing (PT) can monitor and aid in improving quality performance of clinical laboratories and commercial products, PT providers often use altered, in contrast to authentic, human specimens as a matrix. As a result, laboratory performance is often assessed against its peer group mean/median but does not evaluate absolute accuracy of the analytical system. We conducted accuracy-based PT for testosterone, using commutable samples, as a follow up to our previous accuracy-based PT done during Sept 2012-Jan 2013 (data not shown).

**Methods:** Five samples were prepared using single-donor authentic human serum and distributed to NYSDOH-certified laboratories. The samples were analyzed for testosterone using 16 different analytical systems. The target values were determined using the CDC reference measurement procedure.

**Results:** Sixty-five laboratories reported results. Eight of 16 analytical systems had ≥ 3 participants and only their results were examined for analytical system mean and bias of total testosterone (Table). All 65 laboratories' results were evaluated against a single criterion (target ± 25.1%), the minimal requirement for total allowable error based on biological variability. The percentages of results that met the criterion for samples 1 to 5 were 35.4%, 98.5%, 89.2%, 96.9%, 83.1%, respectively. We defined obtaining results for at least 4 of 5 samples within ± 25.1% as "passing." Of all 65 participating laboratories, 87.7% had passing scores. The passage rates for 8 analytical systems are listed in the table (first column from left). Only one analytical system, which had obtained CDC Hormone Standardization (HoST) certification until 2013, had biases < 5% for samples 2-5, with concentrations seen in hypogonadism and normal adult male.

**Conclusions:** Our results indicate that efforts in improving assay accuracy and precision for testosterone assays remain relevant and necessary.

| Sample ID | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Analytical System (n), Passage rate % | Mean ng/dL (Bias, %) | | | | |
| CDC target | 43.5 | 160 | 294 | 457 | 534 |
| Abbott Architect i System (4), 100% | 55.2 (26.8) | 169.5 (6.0) | 294.2 (0.1) | 505.0 (10.5) | 596.2 (11.6) |
| Beckman Coulter Access2 (7), 100% | 79.8 (83.4) | 155.4 (-2.9) | 307.2 (4.5) | 436.1 (-4.6) | 474.4 (-11.2) |
| Beckman Coulter UniCel DxI 600 (9), 78% | 81.5 (87.3) | 152.9 (-4.4) | 288.8 (-1.8) | 410.5 (-10.2) | 429.0 (-19.7) |
| Beckman Coulter UniCel DxI 800 (6), 100% | 82.2 (89.0) | 151.5 (-5.3) | 285.3 (-2.9) | 401.6 (-12.1) | 427.2 (-20.0) |
| Roche Cobas e601 (3), 100% | 65.0 (49.4) | 165.3 (3.3) | 305.9 (4.0) | 479.0 (4.8) | 521.7 (-2.3) |
| Siemens ADVIA Centaur (15), 73% | 53.4 (22.7) | 144.7 (-9.6) | 249.9 (-15.0) | 423.8 (-7.3) | 422.1 (-21.0) |
| Siemens Immulite2000 (8), 100% | 39.8 (-8.5) | 148.3 (-7.3) | 329.6 (12.1) | 438.8 (-4.0) | 414.3 (-22.4) |
| Tosoh Bioscience (4), 75% | 59.7 (37.2) | 148.9 (-7.0) | 357.3 (21.5) | 533.8 (16.8) | 617.3 (15.6%) |

## A-184

### Performance Evaluation of a Total Inhibin ELISA and Reference Intervals in Female and Male Populations

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**Background:** Inhibins are dimeric glycoproteins secreted primarily by the granulosa cells in the ovaries and Sertoli cells in the testes. The hormone consists of an α-subunit linked with either a βA-subunit or a βB-subunit, resulting in heterodimers designated as inhibin A and inhibin B, respectively. Several forms are present in the circulatory system including mature and partially processed αβ-dimers, and inactive free α-subunits. The measurement of inhibins is clinically useful in the diagnosis and prognosis of granulosa cell and mucinous tumors of the ovary. It has been demonstrated that granulosa cell tumors secrete inhibin A, B and the free α-subunit while mucinous tumors primarily secrete the free α-subunit. The purpose of this study was to assess the performance characteristics of and to validate the Ansh Labs (Webster, TX) Total Inhibin ELISA. Additionally, a reference limit for postmenopausal women was verified and reference intervals for premenopausal women and men were established.

**Methods:** Deidentified residual serum specimens sent to ARUP Laboratories for routine testing, as well as serum specimens obtained from healthy volunteers, were used for this study. Total inhibin was measured according to the test kit manufacturer's protocol. The performance characteristics evaluated were analytical sensitivity, linearity, method comparison, precision and analyte stability. Reference limit and interval studies were performed with serum specimens obtained from healthy volunteers. The University of Utah's Institutional Review Board approved this study.

**Results:** The analytical sensitivity was as follows: Limit of blank, 0.3 pg/mL; limit of detection, 2.0 pg/mL; limit of quantitation, 9.0 pg/mL (parametric analysis of 60 zero calibrator, 60 approximately 3 pg/mL and 40 approximately 9 pg/mL measurements; allowable error, 20%). Linearity was established by combining serum specimens with high and low total inhibin concentrations at different ratios to create as set of 9 specimens, each of which were tested in triplicate. Linear regression analysis produced a slope of 1.02, intercept of -15.8 and $r^2$ of 0.997. A method comparison study (n = 40) with another lab using the same total inhibin assay, generated a slope of 1.06, intercept of -6.6, and r of 0.993. Precision was determined from two serum pools of differing total inhibin concentrations tested over 20 days, four replicates per pool per day. Repeatability and within-laboratory CVs were 3.7 and 7.8% at 34.4 pg/mL, and 2.8 and 4.1% at 373.9 pg/mL, respectively. Total inhibin was stable for 12 hours at room temperature, 7 days (min) at 4-8 °C, 3 months (min) at -20 °C, and over a minimum of 3 freeze/thaw cycles. A postmenopausal reference limit of 10 pg/mL was verified (n = 21, 97.5$^{th}$ percentile). Reference intervals were established as 2-300 pg/mL for premenopausal females and 50-190 pg/mL for males (n = 125 each, nonparametric analysis, 95$^{th}$ percentile).

**Conclusions:** The Ansh Labs Total Inhibin ELISA demonstrates acceptable performance for quantifying total inhibin in human serum. Reference intervals have been established for both premenopausal females and males, and a reference limit verified for postmenopausal females.

## A-185

### Unexpected high values of LH: high molecular weight forms (macro LH)?

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**Background:** Several pre-analytical and analytical interference factors that could influence hormone tests and hamper its interpretation have been described. Autoantibodies can cause interference in immunoassays for a number of analytes including insulin, growth hormone, thyroid hormones, prolactin, TSH and most rarely, for luteinizing hormone (LH). **Methods:** We present the case of a female patient, 45 years of age, with a diagnosis of primary hypothyroidism, Hashimoto thyroiditis, when she was 30 years old. She had also multinodular goiter, submitted to total thyroidectomy 12 years ago. The diagnosis was benign, folicular adenoma. She had regular menses. The patient never used any LH-stimulating drug, nor had ever received LH or HCG injections. **Results:** The laboratory evaluation showed a constantly high LH value (>200.0 IU/L, ECLIA, Roche), with FSH levels ranging from 3.2 to 26.5 IU/L; estradiol, 28 to 495 pg/mL; prolactin, 14.3 to 29.7 ng/mL. LH was also measured by ICMA, Advia (Siemens) and Unicel (Beckman), with values of 74.6 and 23.7 IU/L, respectively. Serial dilution showed parallelism with the curve obtained with a standard LH preparation. Antibodies against thyroperoxidase were present in high concentrations, 653 KU/L (reference levels < 35 KU/L). Her serum was subjected to gel-filtration chromatography on a Superdex 200 colunn (0.9 x 30 cm; Pharmacia) calibrated with the Pharmacia high-molecular-weight calibrators, and the elution showed that almost all of the LH eluted as a high-molecular-weight form ($M_r$ >250000). Recovery after precipitation with polyethylene glycol was very low, LH 3.7 IU/L, consistent with a macro LH. **Conclusions:** The etiology of this phenomenon is probably a complex of LH with immunoglobulin (Ig)-G, particularly with anti-LH autoantibodies. The relationship with autoimmune diseases (Hashimoto thyroiditis) remains to be defined. This condition must be considered in the event of a finding of unexpectedely high LH values, non-coincident with the patient clinical context.

## A-186

### Analysis of Anti-Müllerian Hormone Levels in Adult Chinese Women: A Multicenter Reference Intervals Study

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**Background:** Anti-Müllerian hormone (AMH) plays an important role in ovarian reserve assessment and individualized in vitro fertilization (IVF) treatment. Due to the surge in the numbers of women delaying childbearing until older ages and patients with reproductive disorders or premature ovarian failure in Chinese populations, it is urgent to obtain an accurate, representative AMH reference interval for adult Chinese women.

**Methods:** From May to September 2013, sera from 1,169 apparently healthy adult females from five regional representative cities in China (Beijing, Hangzhou, Guangzhou, Dalian and Urumqi) were collected, and we used a Beckman DxI800 automated chemiluminescence immunoassay analyzer to detect AMH levels. A multiple regression analysis was used to investigate the effects of region, sex, age, Body Mass Index (BMI), Systolic Blood Pressure (SBP), exercise on AMH. We evaluated 5 candidate regression models to describe the decline of AMH with age and established AMH reference intervals in different age groups.

**Results:** The main factor affecting AMH levels was age (B = -0.756, P<0.001). Regions, BMI, SBP and exercise had no significant effects on AMH levels. The linear, quadratic and cubic models could either provide the best fit regression model to describe the decline of AMH with age ($R^2$=0.40). The AMH reference intervals for adult Chinese women aged 19~24 years, 25~29 years, 30~34 years, 35~39 years, 40~44 years , 45~49 years and ≥50 years were 0.74~16.06, 0.67~11.64, 0.50~9.99, 0.09~8.33, 0.04~4.09 , 0.01~1.46 and 0.00~0.18 ng/ml, respectively.

**Conclusion:** This study used an AMH chemiluminescence reagent newly developed by Beckman to establish AMH reference intervals for adult Chinese women in different age groups. The results have important reference value for the clinical application of AMH as a biomarker.

## A-187

### Audit Of Oral Glucose Tolerance Testing

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**Background:** In the Singapore Ministry of Health diabetes diagnostic algorithm, oral glucose tolerance tests are restricted to patients with fasting plasma glucose concentrations of 6.1 - 6.9 mmol/L. This guideline was published in 1999 and is the standard of care in Singapore. This study examined whether indeed this algorithm is used in a 1400 bed general hospital and whether the results could be classified into the accepted categories of impaired fasting glycaemia (IFG), impaired glucose tolerance (IGT) and diabetes mellitus (DM). **Methods:** Details of all oral glucose tolerance tests performed from 2013 - 2015 inclusive were extracted from the laboratory information system. An oral glucose tolerance test involves collection of fasting and 120 min plasma glucose samples following a 75g oral glucose load. **Results:** In 3 years, 1125 oral glucose tolerance tests were performed of which 254 (22.6%) had fasting glucoses of 6.1-6.9 mmol/L. The final categorisation for these cases was: 45 IFG, 87 IGT and 121 DM. Comparing the results of the fasting glucose and 120 min glucose for the other cases, there was 81% concordance with 155 fasting glucose <7.0 / 120 min glucose >=11.1 mmol/L and 22 fasting glucose >=7.0 / 120 min glucose <11.1 mmol/L. There were 16 cases with fasting glucose >10.0 mmol/L. There were 76 cases with fasting glucose <=4.5, of which none had 120 min glucose >=11.1 mmol/L. **Conclusion**: Most oral glucose tolerance tests did not meet the Singapore Ministry of Health criteria justifying the performance of an oral glucose tolerance test. In 20% of these cases, the fasting glucose and 120 min glucose results led to conflicting categorisation. Better clinician education and triage of requests is needed to reduce inappropriate requests and diagnostic confusion. As a first step, deciding not to proceed with an oral glucose tolerance test if the fasting glucose concentration <=4.5 mmol/L would reduce unnecessary testing without any potential diagnostic data loss.

## A-188

### Relationships between Vitamin D Status, Androgens and Determinants for Severity and Progression in Some Prostate Diseases

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**Background:** The hypothesis that androgens can cause prostate growth and accelerate prostate cancer is nowadays replaced by the saturation concept. Recently, the antiproliferative effect of calcitriol provoked intensive research on the role of vitamin D in prostate growth and tumor aggressiveness. We aimed to investigate the relationships between vitamin D status, androgens and determinants associated with the severity and progression of benign prostate hyperplasia (BPH) and prostate cancer (PCa).

**Methods:** One hundred twenty five men with clinical suspicion for prostate disease evoked by elevated serum PSA levels and/or abnormal digital rectal examination, consented to enter the study. Patients with acute prostate inflammation, systemic infection, cardio-respiratory failure, diseases contraindicating surgical treatment, and vitamin D supplementation were excluded. According to the biopsy results, patients were divided into two groups: 37 with BPH and 88 with PCa. Tumors were graded by the Gleason grading system. PCa patients were divided into three risk groups (RG) according to EAU guidelines (RG1-low; RG2-intermediate; RG3-high) and by the tumor grade (Gleason score<7:Gl1-low; Gleason score=7: Gl2-intermediate; Gleason score>7: Gl3-high). Total testosterone (TT), free testosterone (FT), dehydroepiandrosterone sulfate (DHEAS), androstendione, and sex-hormone binding globulin (SHBG) were assayed by verified ELISA methods. Free androgen index (FAI) was calculated as TT/SHBG. Vitamin D status was evaluated by the serum levels of 25-hydroxyvitamin D (25OHD) measured by a validated LC-MS/MS method. Prostate specific antigen (PSA) was measured by a standard chemiluminescent immunoassay. GraphPad Prism v.6.00 was used for data analysis: t-test and one-way ANOVA to find mean differences and Spearman's test to establish the correlation of tested parameters.

**Results:** Vitamin D deficiency (<50nmol/L) was more frequent in PCa than in BPH patients (69.4% versus 43.2%). Among measured androgens TT and FT revealed a significant decrease in the PCa group versus BPH, while DHEAS was increased in PCa patients. A significant negative relationship between DHEAS and PSA (r=-0.33, p=0.05), and a tendency for a decrease of 25OHD with the increase of PSA (r=-0.29, p=0.09) were established in BPH. Significant negative correlation with 25OHD was detected only for FAI (r=-0.42, p=0.03). A weak, but significant negative correlation was established between PSA and TT (r=-0.23, p=0.03), FT (r=-0.28, p=0.009), and DHEAS (r=-0.22, p=0.04) in PCa. Stratification by the cut-off value (50nmol/L) for 25OHD showed significantly higher PSA levels for the vitamin D deficient group (20.91±22.31ng/ml vs 27.70±4.28ng/ml, p<0.001). Increase of risk and the tumor aggressiveness was associated with decrease in 25OHD and FAI: a negative correlation with tumor grade and risk was found for 25OHD (r=-0.19, p=0.08; r=-0.23, p=0.05 respectively), and for FAI (r=-0.27, p=0.01; r=-0.25, p=0.03 respectively). ROC curve analysis showed highest discriminative value between BPH and PCa for 25OHD (AUC±SE= 0.68±0.05; CI=0.57 - 0.78; p=0.002).

**Conclusion:** Significant differences between PCa and BPH were observed for all tested steroids. Association with risk and tumor grade, and eventual discriminative potential between BPH and PCa was found for 25OHD.

## A-189

### Development of Novel Specific and Sensitive ELISAs for Proglucagon-Derived Peptides

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**Objective:** The aim of this study was to develop well characterized sensitive and specific ELISAs to quantitate Glucagon, Oxyntomodulin (OXM), and Glucagon-like peptide 1 (GLP-1) in biological fluids. **Relevance**: Proglucagon, (PG) a 160aa peptide is cleaved from preproglucagon and the later is encoded by the glucagon gene (GCG) in humans. PG is a precursor of Glucagon, OXM, GLP-1 and several other peptides. These peptides arise by differential processing of PG. Glucagon, corresponding to PG residues (33-61aa), is formed in the alpha cells of the pancreas. Oxyntomodulin is a 37aa peptide hormone secreted by the gut endocrine L-cells post-prandially and shares identical amino acid sequence in the N-terminal to glucagon, with an extension of 8aa peptide in the C-terminus. Prohormone convertase 1/3 cleaves Proglucagon precursor into Oxyntomodulin, GLP-1/2 and GRPP upon nutrient ingestion. Oxyntomodulin is known to bind both the GLP-1 receptor and the glucagon receptor, but with lower affinity compared to GLP-1 and glucagon. Oxyntomodulin has been studied as a weight loss agent in obese patients via suppression of food intake and increase in energy expenditure. Glucagon has been studied for the treatment of hypoglycemia and glucagon receptor antagonists are under development for the treatment of type 2 diabetes. GLP-1 and GLP-2 receptor agonists appear to be promising therapies for the treatment of type 2 diabetes and intestinal disorders, respectively.

**Methodology:** Specific monoclonal antibody based ELISAs for glucagon (AL-157), oxyntomodulin (AL-139), and GLP-1 (AL-172) have been developed to measure their respective analyte in ≤50uL of the plasma. The glucagon assay is standardized to NIBSC code 69/194 v3.0 preparation and the other assays were gravimetrically calibrated to their corresponding pure peptides. These ELISAs were validated for their specificity to the Proglucagon fragments, specimen stability, and their circulating levels (fasting and non-fasting) in matched serum and plasma. Monoclonal antibody based ELISAs for GRPP, GLP-2, and MPGFs has also been developed and will be presented in the poster.

**Validation:** Glucagon, OXM, and GLP-1 ELISAs with a dynamic range of 20-300pg/mL, 3-300pg/mL, 15-600 pg/mL are highly specific to glucagon, OXM, and GLP-1, respectively. These assays did not cross-react to GRPP, Glucagon, OXM, GLP-1, and GLP-2 when assayed in their individual ELISAs. Proglucagon KO serum samples (n=3) in the OXM assay were non-detectable, whereas a concentration of 103-246pg/mL was observed in the wild type mice (n=3). Median levels of Glucagon, OXN when studied in fresh/2-8ºC/1FT/2FT drawn in EDTA plasma (no DPP-4) were 75.8/82.9/84.4/83.8pg/mL and 353.8/342.4/389.3,409.9pg/mL, respectively. Median GLP-1 level (2 FT) on the same subjects was 235.2pg/mL. Fasting/non-fasting (n=5) median Glucagon, OXN, and GLP-1 levels were 85.1/84.6, 215.3/645.9, 215.7/269.3pg/mL, respectively.

**Conclusions:** Whole portfolio of easily accessible and standardized assays for Proglucagon-derived peptides are available to reliably quantitate these important endocrine and local regulators in physiological and pathophysiological studies for metabolic disorders.

## A-190

### Performance Evaluation of the ADVIA Chemistry Enzymatic Hemoglobin A1c Assay*

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**Background:** According to the World Health Organization, an estimated 422 million adults were living with diabetes globally in 2014. Early diagnosis of diabetes is critical for the management of the disease. The longer a person lives with undiagnosed and untreated diabetes, the worse his or her outcome will likely be. Glycemic states can be measured by fasting blood glucose, serum fructosamine, or glycated hemoglobin (HbA1c). HbA1c is a better indicator of mean blood glucose level. 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 use on the automated random-access ADVIA® Clinical Chemistry Systems. The objective of this study was to evaluate the performance of this new A1c_E assay on the ADVIA Clinical Chemistry Systems.

**Methods:** The first step of the reaction is to hemolyze the red cells with the pretreatment solution and convert hemoglobins to methemoglobin. The first reagent (R1) is added to form azido-methemoglobin, and the protease in R1 hydrolyzes glycated hemoglobin to form fructosyl-valine-histidine. The second reagent (R2) containing fructosyl peptide oxidase is added to convert the fructosyl-dipeptide to $H_2O_2$ (a byproduct of the enzymatic oxidation reaction) that reacts with the chromagen, 10-carboxymethylaminocarbonyl)-3,7-bis(dimethylamino)-phenothiazine (DA-67), in the presence of horseradish peroxidase. The performance evaluation in this study included precision, linearity, correlation with the NGSP reference method, and total error assessment. Data were collected on ADVIA Clinical Chemistry Systems (1800, 2400, and XPT), which use the same reagent packs, calibrators, and commercial controls.

**Results:** The precision (within-lab %CV) of the new A1c_E assay using two levels of commercial controls and five whole-blood pools ranging from ~4.50 to ~12.00% HbA1c (n = 80) on the ADVIA Clinical Chemistry Systems across three lots was ≤1.3% (repeatability) and ≤1.9% (within-lab). The analytical range of the assay was 3.8–14.0% HbA1c. The assay correlated well with the NGSP: ADVIA 1800 A1c_E assay = 1.03 [NGSP] − 0.204 (r = 0.994, n = 163; sample range: 3.70–14.60% HbA1c). The assay demonstrated a %TE ≤3.92 on the ADVIA 1800 Clinical Chemistry System.

**Conclusions:** The A1c_E assay on the ADVIA Clinical Chemistry Systems 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-191

### Performance Evaluation of Free Thyroxine (FT4) and Thyroxine (T4) Assays[1] on the Atellica Immunoassay Analyzer[2]

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**Introduction:** Quantitative measurements of free thyroxine and thyroxine are important for the detection, diagnosis, and treatment of thyroid disease. The prototype Atellica™ IM FT4 and T4 assays[1] (Siemens Healthcare Diagnostics Inc.) are intended for the quantitative measurement of FT4 and T4 using the Atellica Immunoassay (IM) Analyzer.[2] The purpose of this study was to evaluate the analytical performance of the Atellica IM FT4 and T4 assays with serum samples.

**Methods:** The Atellica IM FT4 and T4 assays[1] are "competitive" immunoassays utilizing direct chemiluminescent technology. They use the same reagent formulations as the ADVIA Centaur® FT4 and T4 assays (Siemens Healthcare Diagnostics Inc.). For the Atellica IM FT4 assay, free T4 in the patient sample competes with acridinium ester-labeled T4 in the lite reagent for a limited amount of biotinylated polyclonal rabbit anti-T4 antibody. Biotin-labeled anti-T4 is bound to avidin that is covalently coupled to paramagnetic particles in the solid phase. For the Atellica IM T4 assay, T4 in the patient sample competes with T4, which is covalently coupled to paramagnetic particles in the solid phase, for a limited amount of acridinium ester-labeled monoclonal mouse anti-T4 antibody in the lite reagent. The Atellica IM T4 assay requires an ancillary reagent that contains a releasing agent to free up the bound T4. Performance testing included precision and assay comparison studies. The assay comparison study was conducted according to CLSI EP09-A3, with Deming regression of patient sample results compared with results observed from the ADVIA Centaur Immunoassay System. For assay precision, each sample was evaluated in duplicate twice a day for 20 days according to CLSI guideline EP05-A3.

**Results:** The Atellica IM FT4 assay comparison yielded a regression equation of y = 1.011x − 0.099 ng/dL, with r of 0.997, versus the FT4 assay on the ADVIA Centaur XP System with 119 serum samples ranging from 0.45 to 11.6 ng/dL. The Atellica IM T4 assay comparison yielded a regression equation of y = 1.048x − 0.347 µg/dL, with r of 0.993, versus the T4 assay on the ADVIA Centaur XP System with 141 serum samples ranging from 0.3 to 30 µg/dL. The Atellica IM FT4 assay 20-day precision study yielded repeatability of 1.2 to 4.7% CV and within-lab precision of 2.2 to 6.8% CV over a sample result range of 0.4 to 10.7 ng/dL. The Atellica IM T4 assay 20-day precision study yielded repeatability of 1.8 to 7.2% CV and within-lab precision of 3.9 to 12.6% CV over a sample result range of 1.4 to 26.3 ng/dL.

**Conclusion:** The Atellica IM FT4 and T4 assays tested on the Atellica IM Analyzer demonstrated analytical performance capable of providing accurate and precise measurements of free thyroxine and thyroxine.

[1] In development. The performance characteristics of this device have not been established.

Future availability cannot be guaranteed. [2]Not CE-marked. Not available for sale. Future availability cannot be guaranteed

## A-192

### OPTIMIZING CORTISOL COLLECTION AFTER CORTICOTROPIN STIMULATION: HOW MANY TIMES SHOULD WE COLLECT CORTISOL SAMPLES FOR APROPRIATE EXCLUSION OF ADRENAL INSUFFICIENCY?

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**Background:** The last guidelines on diagnosis and treatment of primary adrenal insufficiency published in 2016 by the endocrine society recommends diagnostic testing to exclude primary adrenal insufficiency (PAI) in acutely ill patients with otherwise unexplained symptoms or signs suggestive of adrenal insufficiency. The society recommends the short corticotropin test (250 mcg) as the "gold standard" diagnostic tool to establish the diagnosis of the disease. Samples for cortisol determination is collected at basal, 30 or 60 min after iv corticotropin stimulation. Peak cortisol levels below 500 nmol/L (18 mg/dL) at 30 or 60 minutes indicate adrenal insufficiency. Objectives: The aim of our study was to examine whether the cortisol stimulation test could be performed with fewer samples without compromising its diagnostic value. **Methods:** We performed a cross-sectional retrospective examination of 75 consecutive indivduals submitted to cortisol stimulation test with corticotropin in the context of adrenal insufficiency investigation. Corticotropin was applied intravenously at a dose of 250 mcg. Blood samples for cortisol were taken at time 0, 30 and 60 minutes. Serum cortisol concentration was tested with the Cobas analyzer electrochemiluminescence immunoassay. A test was considered responsive when peak cortisol at any time ≥ 500 nmol/L (18 mcg/dL). **Results:** Sixty three (84 %) of individuals submitted to the corticotropin test showed a positive response to the stimulus (peak cortisol at any time ≥ 500 nmol/L). Mean age was 35.4 ± 20.5, range 2-79 years, with female:male ratio of 2.6:1. Fifteen (24 %) of individuals were children under 18 year. Median cortisol values at time 0, 30 and 60 were respectively 10.7, 20.4 and 24.5 mcg/dL. All of our responsive individuals showed a peak cortisol response 60 minutes after corticortrophin. **Conclusions:** As cortisol peak happens 60 minutes after corticotropin stimulus and as the diagnosis of PAI is based on the peak stimulated serum cortisol concentration after the stimulus, it seems rational, cost-effective and more comfortable for the patient to collect cortisol only once, 60 minutes after corticotropin administration. We emphasize that such a procedure does not compromise the diagnostic accuracy of the test.

## A-193

### Retrospective analysis of the utility of anti-thyroglobulin antibody testing to assess thyroid autoimmunity

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**Objective**: Antibodies to thyroperoxidase (anti-TPO) and thyroglobulin (anti-Tg) are associated with autoimmune thyroid diseases (AITD) – Hashimoto's thyroiditis and Graves' disease. While elevation of one or both antibodies is associated with AITD, clinical practice guidelines published in 2012 by the American Thyroid Association (ATA) and American Association of Clinical Endocrinologists (AACE) do not recommend measurement of anti-Tg levels in the assessment of patients with suspected or known AITD. Instead, the guidelines advocate for measurement of anti-TPO alone in certain cases of suspected autoimmune hypothyroidism. Despite this, in our practice we have observed that providers frequently order both anti-TPO and anti-Tg to assess patients with known or suspected AITD. Therefore, our objective was to retrospectively assess the diagnostic utility of anti-Tg testing by determining the concordance and discordance of results compared to anti-TPO.

**Methods**: The results of 1204 anti-thyroid antibody tests, performed between 4/1/2016 and 6/30/2016, were retrospectively reviewed. 708 test results represented 354 patients who underwent testing for both anti-Tg and anti-TPO. An additional 477 patients underwent anti-TPO testing alone, and 19 patients had anti-Tg testing alone. Anti-TPO and anti-Tg were measured on the Siemens Immulite 2000 (Siemens Healthcare Diagnostics) by chemiluminescent immunoassays. The reference interval for anti-TPO was < 35 IU/mL, and for anti-Tg was < 40 IU/mL.

**Results**: Out of 354 patients with both anti-TPO and anti-Tg testing, 78% of patients (n= 277) showed concordance for anti-Tg and anti-TPO. Among concordant cases, 11% of patients (n = 40) had elevation of both anti-Tg and anti-TPO, whereas the remaining 67% of patients (n = 237) had anti-ATG and anti-TPO within normal limits. 22% of patients (n = 77) had discordant test results. Out of the discordant cases, 18% (n = 62) had high anti-TPO and normal anti-Tg, whereas only 4% (n = 15) had high anti-Tg and normal anti-TPO. Therefore, testing of anti-TPO alone would have accurately identified the presence or absence of thyroid autoimmunity in 96% (n = 339) of all patients studied in our retrospective cohort.

**Conclusion:** While both anti-TPO and anti-Tg may be elevated in patients with AITD, our data support the ATA/AACE guidelines, which recommend reliance on anti-TPO testing alone for assessment of thyroid autoimmunity. This approach would have eliminated 339 unnecessary anti-Tg tests over a 3 month period, for a projected elimination of 1,356 anti-Tg tests per year. Another consideration is to offer anti-Tg as a reflex test when anti-TPO is negative; however in our cohort of 354 patients, 71% (n = 252) had normal anti-TPO levels, and therefore relying on reflex testing would result in a substantial number of anti-Tg tests still being performed. Additionally, introducing a reflex testing option might influence providers who routinely order anti-TPO alone to select anti-TPO with reflex to anti-Tg, thereby increasing anti-Tg testing. Therefore, we recommend that providers be educated on the low yield of anti-Tg testing, and that they utilize this test only in patients with negative anti-TPO and a strong clinical suspicion for autoimmune hypothyroidism.

## A-194

### Design Optimization for the ADVIA Centaur Anti-Müllerian Hormone Assay*

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**Background:** Measurement of anti-Müllerian hormone (AMH) in vitro has become a significant tool for the assessment of ovarian reserve and an aid in the evaluation of polycystic ovary syndrome. Considering intrinsic and extrinsic factors that may influence AMH levels, an assay that can produce reliable and reproducible results is highly desirable.[1-5] The objective of this study was to design and optimize an AMH assay* from Siemens Healthineers on the ADVIA Centaur® Immunoassay Systems.

**Methods:** A direct sandwich format was selected for the assay. Screening studies were conducted to optimize the performance of the solid phase and the detection ligand. The solid phase optimization included evaluation of commercially available magnetic latex particles (MLPs) precoated with streptavidin and in-house paramagnetic particles (PMPs) pre-coated with anti-fluorescein isothiocyanate antibody. Multiple acridinium ester (AE) labels were evaluated using the same MLP to identify a suitable detection ligand that produces optimal signal-to-noise ratio (S/N). Assay standards and controls were developed utilizing affinity-purified AMH from bovine tissue in protein buffer matrix. In-use stability of targeted AMH doses representing the lyophilized standards and control levels was evaluated at 2-8°C and −20°C after reconstitution. Fractional factorial design of experiment was used to identify the main factors affecting the standard curve slope and the magnitude of signal separation in the assay.

**Results:** The maximum S/N for the MLPs was 841 for Dynabeads M280, followed by S/N of 838 for Dynabeads M270, 589 for Dynabeads MyOne T1, 337 for Dynabeads MyOne C1, and 205 for Agilient LodeStars. All other MLPs and in-house PMPs reported S/N below 150. A double-zwitterionic AE was selected based on the highest S/N in comparison to other hydrophilic AE labels and robust performance during ambient temperature fluctuations study. The S/N obtained with the double-zwitterionic AE and Dynabeads M280 was 1.2-1.6 fold improvement compared to the AE candidate with the lowest signal separation. The average recovery after reconstitution of lyophilized material containing targeted levels of AMH antigen at 14 days (2-8°C) and 30 days (−20°C) was 97% and 90%, respectively. The main factors affecting the S/N were sample volume, detection reagent volume, MLP concentration, and detection antibody concentration.

**Conclusion:** Highest S/N ratios were observed using streptavidin-coated M280 Dynabeads. The charge-neutral double-zwitterionic AE characteristics provided better signal separation in comparison to hydrophilic AEs with modified polyethylene glycol moieties. In-use stability study shows good antigen concentration recovery for up to 30 days. * Under development. The performance characteristics of this device have not been established. Not available for sale and its future availability cannot be guaranteed.

## A-195

### Thyroid function tests in Turkish geriatric population

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**Background**

Subclinical hypothyroidism or hyperthyroidism is a common condition in the older population. The diagnosis of thyroid dysfunction remains challenging in older population, thus based on the measurement of thyroid function tests. To avoid misclassification and potential overestimation of thyroid dysfunction in geriatric patients, age specific reference ranges should be used. The aim of this study was to evaluate FT4, FT3 and TSH reference levels in participants aged ≥65 years.

**Methods**

FT4, FT3 and TSH, anti-thyroglobulin (antiTG) and thyroid peroxidase antibody (antiTPO) levels were measured by DxI 800 (Beckman Coulter Diagnostics, USA). The new set up TSH immunoassay was used in the study which shows better analytical sensitivity at low TSH concentrations, compared to the old method.

Individuals with antiTPO>9 IU/mL and antiTG>4 IU/mL were excluded and 122 individuals over 65 years old without any known thyroid disorder composed the study group.

The statistical analysis was performed by using IBM SPSS software, version 21 (SPSS Inc., Chicago, IL, USA) and MedCalc version 14.8.1 (Mariakerke, Belgium). Statistical significance was assumed when the p-value was <0.05. All results were expressed as mean±standard deviation (SD). Independent sample t test was used for the comparison of TSH, FT4 and FT3 values in gender and age group (65-75 and >76). Outliers were tested with the D'Agostino-Pearson test. The reference intervals were calculated with reference interval for normal distribution.

**Results**

The prevalence of antiTPO positivity was 8.3% and AntiTG positivity was 5.8% in our study group. In 2.5% of the individuals, both antibodies were out of the normal range. Age-specific geriatric reference ranges for TSH, FT4 and FT3 were determined after the exclusion of these individuals. At 2.5$^{th}$ lower limit (CI) and 97.5$^{th}$ upper limit (CI), the age-specific TSH range was 0.33 [0.28 – 0.39] mIU/mL and 3.99 [3.35 – 4.76] mIU/mL, mean±SD was 1.35 ± 0.79 mIU/mL, respectively. For FT4 mean±SD was 12.79±2.49 pmol/L, reference range was 7.86 [7.15 – 8.57] pmol/L and 17.85 [17.14 – 18.55] pmol/L. For FT3, mean±SD was 4.30±0.85 pmol/L, reference range was 2.57 [2.32 – 2,81] pmol/L and 6.02 [5.77 – 6.26] pmol/L. According to the Beckman Coulter system, TSH, FT4 and FT3 reference values for individulas between 18-65 years were 0.38-5.33 mIU/mL, 7.86-14.41 pmol/L and 3.8-6.0 pmol/L, respectively.

**Conclusion**

We observed an age dependent decline in TSH levels in individuals over 65 and also FT4 levels were higher in geriatric individuals when compared with the commercial assay reference range determined by Beckman Coulter. These differences may be due to the differences in the iodine status of the Turkish diet and environmental factors. Before therapy is initiated, thyroid function tests should be repeated in 6 to 12 months to exclude laboratory error or transient elevations.

## A-196

### The Use of Fructosamine in Cystic Fibrosis-Related Diabetes (CFRD) Screening

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**Background:** Cystic fibrosis related diabetes (CFRD) is a disease of transient hyperglycemia, which if unrecognized and untreated results in irreversible decline in lung function and increased morbidity and mortality. Currently, CFRD is diagnosed with the oral glucose tolerance test (OGTT), as traditional markers of glycemic control, such as HbA1c and fasting glucose are unreliable in patients with CF. Given that compliance with the OGTT is poor, and screening thresholds are not based on relevant CF outcomes, such as impaired lung function, there is great interest in identifying an alternate screening test for CFRD. Serum fructosamine is a simple blood test that measures total glycated serum protein, and is used in clinical settings where HbA1c is unreliable. Here, we aim to determine whether serum fructosamine correlates with glycemic control and clinical outcomes in patients being screened for CFRD.

**Methods:** Twenty clinically stable adult patients undergoing annual screening for CFRD with the 75 g 2 hour OGTT were recruited for this study. Patients previously diagnosed with CFRD were excluded. A serum specimen was collected before commencing the OGTT, and fructosamine was measured using the Siemens fructosaminase-based method on the Advia 2400. Total protein was measured using the Siemens Biuret method, also on the Advia 2400. Fractional serum fructosamine (FSF) was calculated as fructosamine/total protein. Lung function was assessed by measuring the percent predicted forced expiratory volume in one second ($FEV_1$) by spirometry. Simple linear regression was performed in Microsoft Excel to assess the correlation between fructosamine and 2 hour OGTT results, FSF and 2 hour OGTT results, and FSF and $FEV_1$. Coefficients of determination were derived from Pearson correlation coefficients. ROC curve analysis was performed in MedCalc, and the Mann Whitney U test was used to assess statistically significant differences between groups.

**Results:** Based on the OGTT results, two patients (10%) had newly diagnosed CFRD, and three (15%) had impaired glucose tolerance (IGT). Serum fructosamine exhibited a significant positive correlation with 2 hour OGTT results ($r^2$=0.2389, p=0.029). Correction for total protein concentration resulted in a stronger correlation between FSF and 2 hour OGTT results ($r^2$=0.3201, p=0.009). ROC curve analysis suggested that FSF can reliably identify patients with an abnormal OGTT (AUC=0.840, p=0.0002), with a cutoff of ≥3.70 µmol/g exhibiting 100% sensitivity and 67% specificity. In addition, FSF exhibited a negative correlation with $FEV_1$ ($r^2$=0.3732, p=0.035). Patients with FSF ≥3.70 µmol/g has significantly lower $FEV_1$ (median 47%) compared to those with FSF <3.70 µmol/g (median 90%; p=0.015).

**Conclusion:** FSF correlated with both OGTT results and $FEV_1$, and reliably identified patients with abnormal OGTT results. This simple blood test shows potential as an effective tool in CFRD screening, and may greatly improve screening compliance.

## A-197

### Performance Evaluation of the ADVIA Centaur Intact PTH Assay* in Intraoperative Patients

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**Background:** The ADVIA Centaur® PTH assay (Siemens Healthcare Diagnostics Inc.) is a two-site sandwich immunoassay using direct chemiluminometric technology. The first antibody in the Lite Reagent is a monoclonal mouse anti-human PTH (N-terminal) antibody labeled with acridinium ester. The second antibody is a biotinylated monoclonal mouse anti-human PTH (C-terminal) antibody that is bound to streptavidin-coated paramagnetic latex particles in the solid phase. The use of two monoclonal antibodies is expected to reduce lot-to-lot variability compared to assays using polyclonal antibodies. The assay is intended for use as an aid in the differential diagnosis of hyperparathyroidism, hypoparathyroidism, and hypercalcemia of malignancy, as well as intraoperatively in patients undergoing parathyroidectomy. **Objective:** To assess the performance of the ADVIA Centaur PTH assay in intraoperative patients. **Methods**: The study assessed the concordance of the ADVIA Centaur PTH assay with commercially available PTH assays approved for intraoperative use in the U.S. Sets of plasma samples were collected from patients undergoing parathyroidectomy and shipped refrigerated to Siemens Healthineers, where they were tested in singleton. Each set included pre- and post-surgery samples. For the primary analysis, successful surgery was defined as surgery resulting in a 50% or greater drop in PTH level from pre-excision to the 10-minute post-excision test results, after the last parathyroid gland excision. Secondary analyses were also conducted, expanding the success criteria to include samples drawn at 10 minutes ±3 minutes from the last excision and adding the criterion that the final PTH measurement must fall in the normal range for the patient. Another analysis looked at each excision individually rather than on a per-surgery basis. **Results:** A total of 46 subjects were enrolled in the study. The primary analysis included the first 30 eligible subjects. These 30 subjects were diagnosed with primary hyperparathyroidism, aged 38 to 79, and four were males. Twenty-six of these subjects had local PTH tested on the IMMULITE® PTH assay (Siemens Healthcare Diagnostics Inc.) and four on a competitor assay. Twenty-nine of the 30 subjects had a successful surgery based on the local PTH results. The percent positive agreement and overall agreement of the primary endpoint were 100.00% (95% CI 91.5 to 100.00) and 96.7% (95% CI 86.4 to 99.3), respectively. For the secondary analyses, all positive agreements were greater than 90%, and all overall agreements were greater than 85%. **Conclusions:** The study showed acceptable concordance between the assays in this intraoperative intact PTH evaluation. *Under FDA review. Not available for sale in the U.S.

## A-198

### Fulvestrant Interference with Six Automated Estradiol Immunoassays and an LC-MS/MS Method: An Analytical and Clinical Investigation

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**Background:** Fulvestrant is a structural estradiol (E2) analog and selective estrogen receptor downregulator (SERD) used to treat hormone positive (HR+) metastatic breast cancer (MBC) in postmenopausal women. E2 measurements may guide fulvestrant treatment as it is most effective in low E2 environments. The structural resemblance of fulvestrant to E2 raised concerns regarding interference with E2 testing but, to our knowledge, only a single case report has been documented. Unlike immunoassays, LC-MS/MS methods measure mass-to-charge ratios and are presumably not subject to the same interferences.

**Objectives:** Assess fulvestrant interference with six automated E2 immunoassays and an LC-MS/MS method, using spiked serum pools and MBC patient samples.

**Methods:** Fulvestrant interference was evaluated using an in-house LC-MS/MS E2 method and six commercial E2 immunoassays: ARCHITECT ci8200 (Abbott), DxI 800 (Beckman), cobas 8000 (Roche), Advia Centaur and Immulite 2000 (Siemens) and LIAISON XL (DiaSorin). Nine serum pools of different fulvestrant/E2 concentrations were prepared by adding 0.2-1% (v/v) fulvestrant stock (AstraZeneca) to pools of residual serum samples with comparable E2 concentrations as determined by LC-MS/MS. Interference studies were performed at three E2 concentrations (25pg/mL, 50pg/mL, 200pg/mL) and three fulvestrant concentrations (10,000pg/mL, 25,000pg/mL, 50,000pg/mL). Additionally, serum from five postmenopausal women undergoing fulvestrant treatment for MBC was collected prior to intramuscular dosing. Samples were measured on the same day in duplicate on all assays. Fulvestrant interference was determined as percent change and percent cross-reactivity.

**Results:** Biases of -17.4 to 68% percent were observed when comparing immunoassay and LC-MS/MS results for neat specimens at the lowest E2 concentration (25pg/mL), with LIAISON showing the least bias (-3.3%). The spiked pool with E2 concentrations representative for postmenopausal women (25pg/mL) treated with 25,000pg/mL fulvestrant (maximum reported in vivo concentration) showed the largest percent change (spiked vs. neat) for Centaur (544.7%) followed by LIAISON (148.1%), Immulite (140.4%), ARCHITECT (116.7%), cobas (81.1%) and DxI (39.4%). The magnitude of the interference was proportional to fulvestrant concentrations for all E2 immunoassays investigated and was significantly lower at high E2 concentrations. The E2 concentrations determined by LC-MS/MS in the five MBC patient samples ranged from 3.1-10.1 pg/mL, values below the functional sensitivity of 5/6 immunoassays

investigated. The immunoassays measured E2 values of 117.6-193.9pg/mL (Centaur), 53.2–112.0pg/mL (Immulite), 47.0-72.0pg/mL (ARCHITECT), 28.4-48.9pg/mL(LIAISON), 10.3-31.1pg/mL (cobas)

and 5.0-37.0ng/mL (DxI) in the MBC patient samples, representing 49–5,611% difference from LC-MS/MS results.

**Conclusions:** These interference studies expand upon field safety notices issued by several vendors by including clinically relevant E2 concentrations and 3 different fulvestrant concentrations, performed on commercially available platforms on the same day. Centaur and Immulite were the most sensitive to fulvestrant interference, whereas DxI and cobas exhibited the smallest interference. The most significant interference was observed at the lowest E2 concentrations, where clinical decisions are most relevant for this patient population. Importantly, falsely elevated E2 concentrations compared to LC-MS/MS results were observed for all five MBC patient specimens using all six immunoassays, thus LC-MS/MS is the preferred method for this population. This study highlights the importance of characterizing method-specific interferences that may impact treatment decisions.

## A-199

### Performance Evaluation of Free Triiodothyronine (FT3) and Triiodothyronine (T3) Assays* on the Atellica Immunoassay Analyzer**

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**Introduction:** Quantitative measurements of free triiodothyronine (FT3) and triiodothyronine (T3) are important for the management of thyroid functions. The prototype Atellica™ IM FT3 and T3 assays* (Siemens Healthcare Diagnostics Inc.) are intended for the quantitative measurement of FT3 and T3 using the Atellica Immunoassay (IM) Analyzer.** The purpose of this study was to evaluate the analytical performance of the Atellica IM FT3 and T3 assays with serum samples. **Methods:** The Atellica IM FT3 and T3 assays are "competitive" immunoassays utilizing direct chemiluminescent technology. They use the same reagent formulations as the ADVIA Centaur® FT3 and T3 assays (Siemens Healthcare Diagnostics Inc.). For the Atellica IM FT3 and T3 assays, the solid phase employs paramagnetic particles covalently coupled to a T3 analog that competes with free T3 in the sample for a limited amount of acridinium ester-labeled monoclonal mouse anti-T3 antibodies in the Lite Reagent. The ADVIA Centaur T3 assay requires an ancillary reagent that contains a releasing agent to free up the bound T3. Performance testing included precision and assay comparison studies. The assay comparison study was conducted according to CLSI EP09-A3, with Deming regression of patient sample results compared with results observed from the ADVIA Centaur Immunoassay System. For assay precision, each sample was evaluated in duplicate twice a day for 20 days according to CLSI guideline EP05-A3. **Results:** The Atellica IM FT3 assay comparison yielded a regression equation of $y = 0.988x - 0.06$ pg/mL, with r of 0.999, versus the FT3 assay on the ADVIA Centaur XP system with 139 serum samples ranging from 0.67 to 18.9 pg/mL. The Atellica IM T3 assay comparison yielded a regression equation of $y = 1.009x + 0.005$ ng/mL, with r of 0.988, versus the T3 assay on the ADVIA Centaur XP System with 137 serum samples ranging from 0.70 to 7.89 ng/mL. The Atellica IM FT3 Assay 20-day precision study yielded repeatability of 0.9 to 1.9% CV and within-lab precision of 1.3 to 3.3% CV over a sample result range of 2.40 to 19.74 pg/mL. The Atellica IM T3 assay 20-day precision study yielded repeatability of 1.5 to 5.4% CV and within-lab precision ranging of 6.4 to 10.0% CV over a sample result range of 0.5 to 8.0 ng/mL. **Conclusion:** The Atellica IM FT3 and T3 assays tested on the Atellica IM Analyzer demonstrated analytical performance capable of providing accurate and precise measurements of free triiodothyronine and triiodothyronine. * In development. The performance characteristics of this device have not been established. Future availability cannot be guaranteed.** Not CE-marked. Not available for sale. Future availability cannot be guaranteed.

## A-200

### A high-throughput test for diabetes care: an evaluation of the next generation Roche Cobas c 513 hemoglobin A1c assay

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**Objectives:** To evaluate the performance of the Roche Cobas c 513 (Roche Diagnostics, Basel, Switzerland), a next generation immunoassay analyzer, in comparison to the Roche Cobas Integra 800 CTS for HbA1c measurement.

**Introduction:** Diabetes mellitus is a condition that affects all age groups worldwide and can be readily diagnosed using laboratory methods. Monitoring patients with diabetes has always been a challenge for clinicians. Glycated hemoglobin A (HbA1c) levels in blood reflect the mean plasma glucose for the previous 3-4 months. As well, HbA1c has been shown to be the preferred marker for diabetes diagnosis and treatment. A rapid and accurate HbA1c method is of great importance to large clinical laboratories.

**Methods:** The c 513 (Roche Tina-Quant® HbA1c Gen.3 immunoassay) was evaluated against the Integra 800 CTS. Leftover EDTA whole blood specimens containing different hemoglobin species as well as control materials from Roche and Bio-Rad (Bio-Rad, Hercules, California) were used in this study. The evaluation was performed according to CLSI guidelines. Accuracy was determined by measuring samples with %HbA1c values assigned by the College of American Pathologists. The lower limit of measurement for both hemoglobin and HbA1c was calculated as the mean plus 3 SD for a saline blank. The upper limit of linearity was verified using calibrator material or a high patient sample with appropriate dilutions. A method comparison between the c 513 and the Integra 800 CTS was performed by measuring 40 leftover specimens that span the analytical measuring range of the assay. The interference from hemoglobin variants was investigated by measuring samples with one hemoglobin variant on the c 513, the Integra 800 CTS, and the Bio-Rad Variant II Turbo 2.0 systems. To assess the effect of not mixing the specimens prior to analysis, unmixed samples (stored undisturbed at room temperature for up to 24 hours after the initial mixing) were measured.

**Results:** Within run precision was 0.5-0.7%CV for %HbA1c values of 5.6 and 10.6-10.8. Between run precision was 0.8-1.3%CV for %HbA1c values of 5.4, 9.1-9.3, and 13.8-14.4%HbA1c. Accuracy, determined using stored proficiency survey samples, demonstrated an average bias of -1.9%. The lower limits of the hemoglobin and HbA1c measurements were 0.19mmol/L and 0.019 mmol/L, respectively. The upper limit of linearity was 17.0mmol/L and 1.72mmol/L for the hemoglobin and HbA1c, respectively. The c 513 correlated well the Integra 800 CTS (coefficient=0.997, slope=0.93, and *y*-intercept=0.49). Overall, the effect of hemoglobinopathies on this assay was negligible except for specimens containing ≥10%HbF that demonstrated a negative bias. Over a 24 hour period, not mixing the specimens prior to analysis demonstrated a relative bias of -1.9 to 2.7%. The c 513 instrument can process approximately 340 samples per hour, 3.4-fold higher throughput than that of the Integra 800 CTS.

**Conclusions:** Cobas c 513 is a precise and accurate automated analyzer for measuring HbA1c. The major advantage of this instrument is its high throughput capable of testing >7500 specimens in 24 hours or 2500 per shift, making it an ideal choice for large laboratories.

## A-202

### The differential diagnosis and interpretation of discrepant results of thyroid function tests

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**Background**

Most thyroid function tests (TFTs) are straightforward to interpret the clinical impression of euthyroidism, hypothyroidism or hyperthyroidism. Some TFTs, however, such as decreased free T4 (FT4) and normal TSH levels could make a difficulty to differentiate among assay interference, thyroxine replacement therapy, TSH-secreting pituitary adenoma and non-thryroidal illness. This study investigated the incidence for TFT patterns grouped by the FT4 and TSH levels in general hospital and to find the possible causes of discordant TFT patterns according to the characteristics of patients by the comparison among the referral departments.

**Methods**

From August 2015 to August 2016, 22,298 TFTs were performed using MODULAR ANALYTICS E170 immunoassay analyzers with Elecsys FT4 II and Elecsys TSH reagents (Roche Diagnostics, Germany) by the department of laboratory medicine, Konkuk University Hospital, Seoul, Korea. We classified TFT results into seven patterns according to the FT4 and TSH levels using the manufacturer's suggested reference ranges and looked into the incidences in each TFT pattern. The proportion of decreased FT4 and normal TSH among the referral departments was investigated.

**Results**

The incidences in seven TFT patterns in 22,298 TFTs were as follows: 62.7% (13,975 with normal FT4 and normal TSH), 11.9% (2,646 with normal FT4 and increased TSH), 9.6% (2,150 with increased FT4 and decreased TSH), 6.3% (1,405 with normal FT4 and decreased TSH), 3.6% (792 with increased FT4 and normal or increased TSH), 3.1% (695 with decreased FT4 and normal or decreased TSH) and 2.9 % (635

with decreased FT4 and increased TSH). The proportion of decreased FT4 and normal or decreased TSH pattern of 3.1% (695 among 22,298 TFTs) was reclassified based on the referral departments: 10.4% (15/144, Neurosurgery, Odds ratio 5.43 by the comparison with the other referral departments, *P* value < 0.0001), 8.5% (33/389, Psychiatry), 7.4% (23/312, Nephrology), 5.0% (54/1074, Emergency medicine), 5.0% (7/141, Neurology), 4.8% (23/476, Orthopedics), 4.5% (29/652, Gastroenterology), 4.2% (9/215, Hematology & oncology), 4.1% (50/1228, Otorhinolaryngology), 2.7% (111/4058, Endocrinology), 1.2% (62/5116, Surgery) and 2.1% (178/8,493, Other referral departments).

**Conclusion**

When TFTs were classified into the seven patterns according to the FT4 and TSH levels, the incidence of discordant TFT patterns such as decreased FT4 and normal or decreased TSH pattern (3.1%) and increased FT4 and normal or increased TSH pattern (3.6%) was to be remarkable. As a result of classifying the TFT results based on the referral departments, the proportion of decreased FT4 and normal or decreased TSH was significantly varied according to the referral departments. This result suggests that the possibility of discordant TFT results is more likely to be attributed to patient factors rather than to assay errors. Further study should be conducted to investigate additional factors needed to discriminate the various patient factors.

## A-203

### A Comparison of Human Chorionic Gonadotrophin Beta-subunit Measurements Using Three Different Assays for the Early Detection of Pregnancy

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**Background:** Human chorionic gonadotropin (hCG) is a hormone produced by the placenta shortly after blastocyst implantation. hCG consists of two subunits: the common α(alpha)-subunit which is virtually identical to that of luteinizing hormone, follicle-stimulating hormone and thyroid-stimulating hormone; and the β(beta)-subunit which has unique structure and is distinguishing for hCG. Most laboratory pregnancy tests employ monoclonal antibody specific to the β-subunit of hCG (β-hCG) to reduce cross-reactivity with other hormones mentioned above. This study aims to compare the measurements of β-hCG in human serum using three different assays that are commonly used in the qualitative and quantitative pregnancy tests.

**Methods:** Forty-nine patient serum samples requested for hCG testing in the Khoo Teck Puat Hospital (KTPH) laboratory were randomly selected and tested qualitatively using the QuickVue+ One-Step hCG Combo Test Kit (Quidel Corporation, USA) (hereafter called "QuickVue+ assay"). These samples were also quantitatively measured using the Elecsys HCG+β assay on the MODULAR ANALYTICS E170 (Roche Diagnostics, Switzerland) used in the KTPH laboratory (hereafter called "Elecsys assay') and the Access Total βhCG assay performed on the DxI-80 0 analyzer (Beckman Coulter, Brea, CA) used in the National University Hospital Referral Laboratories Pte. Ltd. (NRL), Singapore (hereafter called "Access assay"). The Elecsys assay uses only mouse monoclonal anti-hCG, whereas a combination of rabbit anti-hCG, mouse monoclonal anti-hCG and goat anti-mouse IgG is used as the capture and tracer antibodies in the Access assay. Aside from the β-hCG, intact hCG and nicked forms of hCG, the Elecsys assay also recognizes β-core fragments that yield no detectable response in the Access assay. The Elecsys and Access assay have been standardized against the 4th IS NIBSC code 75/589 and 5th IS NIBSC code 07/364, respectively.

**Results:** Five negative and forty-four positive results were observed from the QuickVue+ assay. The QuickVue+ assay demonstrated 100% clinical sensitivity and specificity as compared to the Elecsys assay (positive pregnancy cut-off at ≥7IU/L in KTPH), and 100% clinical specificity and 95.65% clinical sensitivity when compared to the Access assays (positive pregnancy cut-off at ≥6.1IU/L in NRL). Method comparison between the two quantitative assays yielded a relationship of y=0.76x-45.79 with $R^2$=0.982. Results measured by the Elecsys assay varied from the Access assay by -9.1% to -45.0%, with an overall significant bias of -25.4% ($p<0.05$).

**Conclusion:** Results from the qualitative pregnancy test (based on single-step lateral flow immunochromatographic assay) correlated well with quantitative hCG tests (based on two-step chemiluminescence immunoenzymatic assay). Our data showed that the Quickvue+ assay could detect positive results in specimens containing as low as 7IU/L hCG, which is lower than the 25IU/L hCG claimed by the manufacturer. Poor correlation was observed between the two quantitative assays for β-hCG measurement; this could be due to the differences in method principles and assay standardization. In conclusion, our study found that these three different assays demonstrated comparable efficiency for early detection of pregnancy. Nevertheless, it is important that the results should always be assessed in conjunction with the patient's medical history and clinical findings for accurate diagnosis.

## A-204

### Assessment of HbA1c Levels in Non-diabetics with Hemoglobin E (Heterozygous E or Homozygous E)

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**Background:** To assess the HbA1c levels in non-diabetic Hb E patients.

**Methods:** Subjects from antenatal care and thalassemia screening programs (n=180) underwent Oral Glucose Tolerant Test (OGTT) and their HbA1c were measured. Subjects with iron deficiency (ferritin <30 µg/dl), blood sugar at 2 hr OGTT > 200 mg/dl, regular blood transfusion and previously diagnosed diabetes were excluded. HbA1c was measured using ion exchange HPLC and an enzymatic assay.

**Results:** The mean HbA1c in heterozygous E (EA) from ion exchange HPLC and Enzymatic Assay were 5.63 (0.55) and 5.29 (0.37) respectively; and the mean HbA1c in homozygous E (EE) from ion exchange HPLC and Enzymatic Assay were 3.37 (0.69) and 4.91 (0.28) respectively. HPLC showed more variation than Enzymatic Assay.

**Conclusion:**

Enzymatic HbA1c assay is an appropriate method for measuring HbA1c in hemoglobin E patients and the results of this study are useful for early diagnosis and monitoring diabetes in Hb E.

## A-205

### Validation of the conversion factor between Activity Assays and direct Immunoassay for Plasma Renin

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**Background:** Plasma renin activity(PRA) and the direct renin concentration measured by immunoassay (Ir-PRC) are the methods used for the clinical assessment of primary and secondary Aldosteronism. Favorable and unfavorable factors are found in both assays. PRA is traditionally used, however labor-intensive and requires a great deal of time. While activity assays measure only active renin, direct renin immunoassays measure both active and non-active renin. The automated direct immunoassays stand out for its fast results, however, similarly PRA, conditions such as pregnancy, glucocorticoid excess, estrogen administration overestimation renin. A conversion factor between PRA and Ir-PRC results can be used, but these factor may change according to the method. The objective of our study was to perform an in-house validation of conversion factor 12 between PRA by Elisa-LDN and Liaison direct renin immunoassay (Diasorin) described in literature.

**Methods:**We selected 81 patients, 34 male (age 11-69 years) and 47 female(age 15-85 years). Measurement of renin was performed in both assays. PRA by Elisa-LDN (functional sensitivity 0.14ng/ml.h, range 0.06-4.69 and within-run precision CV 7.2%, inter-assay precision CV 5.67%, reference value 0.2- 3.3 ng/ml.h). In this assay, the plasma sample was aliquoted and the fractions were incubated at 0-4°C and 37°C respectively for 120 minutes, to allow the generation of Angiotensin-I (Ang-I). The PRA were calculated by the Ang-I difference of the sample at 37° and 0-4°. The same samples were analyzed by automated Liaison direct renin immunoassay (Diasorin), (functional sensitivity 1,96 µUI/mL, range 4.4-46.1 and within-run precision CV 3.31%, inter-assay precision CV 8.30% reference value 2.8-39.9 µUI/mL), the results were divided by 12(conversion factor). For statistical analysis were used the Pearson correlation coefficient.

**Results:** 63 results (78%) were in between reference range in both methods, 12 (15%) above the reference value and only 6 (7%) did not correlate. Among the results that not correlate all then had PRA above reference values. The Pearson correlation coefficient was r=0.946 slope 0.8 and intercept 0.6; Among men, r= 0.985 slope 0.7 and intercept 0.6, women r=0.937 slope 0.9 and intercept 0.2

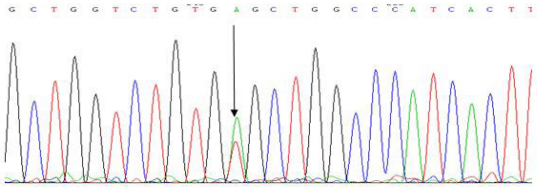
**Conclusion:** The Liaison direct renin immunoassay (Diasorin) has a good correlation with PRA by Elisa-LDN when used conversion factor 12 as already described in the literature.

## A-206

### Effects of Hemoglobin Newyork Traits on Measurements of HbA1c by 11 Methods

D. Wen, W. Wang, S. Xu, M. Suo. *Zhongshan Hospital Affiliated to Sun Yat-sen University, Zhongshan city,Guangdong Province, China*

**Background**: HbA1c is an important tool for monitoring glucose levels. Hemoglobin variants affect some HbA1c tests . Hb New York is a common β-chain variant in southern China. We aimed to evaluate the interference of Hb New York on 11 HbA1c analytical systems, including IE-HPLC (Biorad VARIANT II, Biorad VARIANT II Turbo, Biorad VARIANT II Turbo2.0, Biorad D10, Mindray H50 ), AC-HPLC(Primus Ultra2 , Premier Hb9210), Immunoturbidimetric (Roche PPI ,Cobas c501), CE(Capillarys 2FP)and Enzymatic (Leadman ) methods . **Methods** 141 samples were included in the study categorized as control (homozygous for HbA; n=120,45 diabetes patients , 75 healthy adults) and Hb New York group(heterozygotes for Hb New York; n=30). Primus Ultra2 was used as comparative system. Deming regression analysis was used and ± 10% bias at 6% and 9% was used as limits to evaluate whether Hb New York had significant interference. **Results** The differences of the 95%CI between the 10 systems and the comparative system in control group were within ±0.70%, bias% were less than 6%, the test results were of no statistically significant difference (P >0.05). In Hb New York group, the differences of 95%CI between the results measured by Biorad VARIANT II,VARIANT II Turbo2.0,D10,Mindray H50 ,Premier Hb9210,Roche PPI,Cobas c501,Capillarys 2FP and the comparative system were all within ±0.7%, bias were less than 6%, the test results were of no statistically significant difference (P >0.05). The differences of the 95%CI between the VARIANT II Turbo and Leadman were outside ± 0.7%, bias % were -4.4% ~ -25.3% and -6.2% ~ -31.6% ,the differences were statistically significant (P <0.001). At 6% and 9%, the mean differences of the results were all greater than the clinical acceptable range; **Conclusion** Hb New York interfered with VARIANT II Turbo and Leadman systems. (Figure1).For Hb New York carriers, we suggest using other methods or indicators to monitor glucose levels.



Figurel1: The gene sequencing map of Hb New York

## A-207

### A Comparative Effectiveness Analysis of Three Continuous Glucose Monitors: Guardian, G5, and Libre

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OBJECTIVE:

Self-monitoring blood glucose (SMBG) with a traditional glucose meter often misses peak post-prandial glucose and hypoglycemia. Currently, continuous glucose monitoring (CGM), which determines diurnal blood glucose patterns on a continuous basis, is being introduced to identify fluctuations and trends of blood glucose levels as soon as possible. This study was aimed to compare the accuracy of three continuous glucose monitoring (CGM) devices in subjects with normal glucose tolerance, type 1 and type 2 diabetes mellitus.

RESEARCH DESIGN AND METHODS:

Nine subjects with normal glucose tolerance (age 23 to 58 years), 9 subjects with type 1 diabetes mellitus (age 27 to 58) and 9 subjects with type 2 diabetes mellitus (age 20 to 67) participated in 96-hour closed-loop blood-glucose control experiments. Capillary blood glucose (BG) obtained 7 times a day were paired in time with corresponding CGM glucose (CGMG) measurements obtained from three CGM devices, the Guardian (Medtronic), G5 (DexCom), and Freestyle Libre (Abbott Diabetes Care) worn simultaneously by each subject. Errors in paired BG-CGMG measurements and data reporting percentages were obtained for each CGM device.

RESULTS:

The accuracy of each device did not change for 5 days. Compared with capillary BG reference readings, the G5 showed the lowest mean absolute relative difference (MARD), with 9.1% overall and 18.1% in the hypoglycemia range. For the Guardian and the Libre, MARD was 16.9%/32.2% and 11.7/14.2%, respectively. Also, the mean and SDs for all BG-ARD pairs associated with BG values within 70-300 mg/dL was lowest in the Libre (6.9±1.5) among 3 devices, indicating higher precision of the Libre. Regarding sensor to sensor variability, the SD for the Guardian was the highest, 14.3%. The Libre and G5 comparable results (6.9% and 8.7%, respectively).

CONCLUSIONS:

This study with three CGM devices for BG values from 35 to 544 mg/dL revealed several differences in performance characteristics that include accuracy, precision and reliability. The G5 and Libre showed comparable accuracy and precision, of which the G5 showed the best accuracy and the Libre showed the best precision.

Acknowledgement:

The research resource was provided by the Clinical evaluation of a small body-attached continuous blood glucose monitoring system with automatic calibration function(NRF-2015M3D5A1065857) through of the MSIP, Korea

## A-208

### Performance of Unicel DXI 800 for 25 (OH) Vitamin D Measurement

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**Background:** Vitamin D is actually a fat-soluble prohormone steroid that has endocrine, paracrine and autocrine functions. It is not only important in bone metabolism, but also suggested to have etiological roles in cancer, diabetes, neurological and autoimmune diseases. Vitamin D deficiency is a common health problem worldwide. Measurement of serum 25-hydroxy- vitamin D [25 (OH) D]) is necessary to reveal vitamin D status. Due to its hydrophobic character and strong protein binding, measurement of 25(OH) D is technically demanding. We evaluated the analytical performance of Unicel DXI 800 for 25 (OH) D measurement. Pregnancy and high procalcitonin samples were also used to study the effect of vitamin D binding protein (DBP) concentration over DXI 800 assay performance.

**Methods:** Blood samples were collected from healthy volunteers, pregnants (n=30) and cases with high procalcitonin (n=35) into vacutainer tubes with gel seperator (Becton Dickinson, NJ, USA). All analyses were performed at the Marmara University Pendik R&E Hospital Biochemistry Laboratory with Beckman Coulter immunoassay (DXI 800, CA, USA) and Roche immunoassay (Roche Modular autoanalyzer, Mannheim, Germany) and precision, and correlation studies were performed according to 'Approved Guideline' (EP09-A2).

**Results:** For Beckman Coulter immunoassay, within-run imprecisions for 9 and 47 µg/L were 7.5% and 5.6% and and between-run imprecisions for the same concentrations were 17.8% and 6.6%, respectively. Same blood samples were studied with 2 methods. The median (min-max) for Beckman Coulter immunoassay was 22.1 µg/L (4.1-137.4) (n=40 and for Roche immunoassay was 28.8 µg/L (3-130.3) (n=40). All assays were lineer up to 70 µg/L. Lineer regression analysis were performed and there were no significant deviation from linearity. For the effect of different concentrations of DBP, cases with high procalcitonin and pregnant cases were used. Procalcitonin levels range between 18-85 ng/mL and the median (interquartile range) of vit D levels measured by Beckman Coulter immunoassay was 6.4 µg/L (3-7.7) and Roche immunoassay 4.7 µg/L (4.4-8.8). The median (interquartile range) of vit D levels measured by Beckman Coulter immunoassay for pregnants was 6 (4.4-10) and Roche immunoassay 5.8 (3.7-10). There were significant deviations from linearity between two methods in both high procalcitonin and pregnant cases (P<0.001).

**Conclusion:** DBP levels increase by up to 50% in a high-estrogen state, such as pregnancy, and decrease in certain disease states like systemic inflammation. Laboratorians can select the method they need according to their technical utilities and turnaround times. Each method should be verified by the users according to laboratory settings.

## A-209

### Reference values for serum AMH test in Turkish women

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**Background:** Anti-Müllerian hormone (AMH) is a glycoprotein with well-known roles in growth and differentiation on reproductive system. Current practices in the evaluation of fertility status of women include analysis of AMH levels, as it is

produced by granulosa cells of pre-antral and antral follicles in ovaries. As an ovarian reserve biomarker, serum AMH levels are closely related with several factors such as age and race. Reference values of Roche serum AMH test which were obtained from a worldwide multicenter study are presented as percentiles rather than intervals for narrow age groups. However, we have observed a tendency among AMH test results to be frequently for lower percentiles in reproductive age during our daily practice. This observation implied that Turkish women may have a tendency to have lower serum levels of AMH. The aim of our study was to determine reference values by indirect method from our retrospective data and compare with manufacturer's results of multicenter reference range study. **Methods:** Medical records of female patients from 20 to 50 years of age within the dates of February 2015February 2017 with AMH results were obtained from LIS. Serum AMH analyses were performed with Roche Cobas e601. Outliers from raw data were excluded by Tukey method. 17,571 test results were used for calculation of reference values. Age groups were determined as 20-24, 25-29, 30-34, 35-39, 40-44, 45-50. For each age group, values of 2.5th, 5th, 50th, 95th, 97.5th percentiles were calculated. Statistical analyses were performed with R 3.3.2.**Results:** Reference values calculated were given in Table 1. All percentile values were 23 to 95% lower than manufacturer's values. The most remarkable difference was observed at 2.5th percentile.

**Conclusion:** Our results show that reference values specific for Turkish female population is required. A nationwide study can be planned for more valid results.

| | Age Groups | AMH (ng/mL) Percentiles | | | | |
|---|---|---|---|---|---|---|
| | | 2.5th | 5th | 50th | 95th | 97.5th |
| Roche | 20-24 | 1.22 | 1.52 | 4.00 | 9.95 | 11.70 |
| | 25-29 | 0.89 | 1.20 | 3.31 | 9.05 | 9.85 |
| | 30-34 | 0.58 | 0.71 | 2.81 | 7.59 | 8.13 |
| | 35-39 | 0.15 | 0.41 | 2.00 | 6.96 | 4.49 |
| | 40-44 | 0.03 | 0.06 | 0.88 | 4.44 | 5.47 |
| | 45-50 | 0.01 | 0.01 | 0.19 | 1.79 | 2.71 |
| Düzen | 20-24 | 0.08 | 0.22 | 2.94 | 7.67 | 8.59 |
| | 25-29 | 0.07 | 0.16 | 1.89 | 6.03 | 6.58 |
| | 30-34 | 0.03 | 0.07 | 1.26 | 4.10 | 4.55 |
| | 35-39 | 0.02 | 0.04 | 0.67 | 2.36 | 2.60 |
| | 40-44 | 0.02 | 0.02 | 0.32 | 1.25 | 1.39 |
| | 45-50 | 0.01 | 0.02 | 0.11 | 0.50 | 0.57 |

## A-210

### Diagnosing Gestational Diabetes Mellitus with the Preferred and Alternate Testing Approaches from the Canadian Diabetes Association

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**Background:** Increased maternal and perinatal morbidity is associated with untreated gestational diabetes mellitus (GDM). In 2013, the Canadian Diabetes Association (CDA) published a preferred and alternate approach for the screening and diagnosis of GDM. The preferred 2-step approach begins with a 50 g glucose challenge test (GCT) in a non-fasting state with plasma glucose (PG) measured 1 hour later. A PG ≥7.8 mmol/L is considered a positive screen and a 75 g oral glucose tolerance test (OGTT) is required for GDM diagnosis. A PG ≥11.1 mmol/L is diagnostic for GDM and does not require a 75 g OGTT for confirmation. GDM is diagnosed if *N*≥1 75 g OGTT result is abnormal (fasting ≥5.3 mmol/L; 1 hour ≥10.6 mmol/L; or 2 hour ≥9.0 mmol/L). The alternate 1-step testing approach deploys a 75 g OGTT only (with no prior 50 g GCT). GDM is diagnosed with this approach if *N*≥1 OGTT result is abnormal (fasting ≥5.1 mmol/L; 1 hour ≥10.0 mmol/L; or 2 hour ≥8.5 mmol/L). **Objectives:** Identify the prevalence of GDM diagnosed by the CDA preferred and alternate approaches to testing within our community-based patient population. Quantify the 75 g OGTT confirmation rate for positive 50 g GCT screens within the CDA preferred testing approach. **Methods:** The PG results from all woman who received 50 g GCT and 75 g OGTT from our regional reference laboratory between Jan 2014 and Dec 2016 were retrospectively reviewed. Patients with both 50 g GCT and 75 g OGTT testing from our laboratory were identified through their date-of-birth and health card number. **Results:** *N*=50866 women received 50 g GCT only. 78.5% (*N*=39935) had a negative 50 g GCT screening result (PG <7.8 mmol/L). 1.3% (*N*=659) had a PG diagnostic for GDM (≥11.1 mmol/L). 20.2% (*N*=10272) of patients had a positive 50 g GCT screen and required 75 g OGTT confirmation testing. 21.6% (*N*=2161) of these women did not receive this testing from our laboratory. 75 g OGTT for patients with a 50 g GCT result between 7.8 and 11.0 mmol/L (*N*=8111) confirmed the diagnosis of GDM in 18.7% (*N*=1518) of these women. *N*=7804 women received 75 g OGTT only. This 1-step approach confirmed the diagnosis of GDM in 24.9% (*N*=1944) of patients. **Conclusion:** The majority of pregnant women received the CDA preferred approach to GDM diagnosis and had a negative 50 g GCT screening result (no 75 g OGTT required). The positivity rate for the 50 g GCT screen within our patient population was ~20% but follow-up 75 g OGTT only confirmed a GDM diagnosis in ~19% of these patients. In total, the CDA preferred 2-step approach confirmed the diagnosis of GDM in ~4% of all patients tested. GDM was diagnosed in ~25% of patients who received the alternate 1-step 75 g OGTT testing. This study provides evidence of the benefits associated with the CDA preferred testing approach. It may also serve as an aid for physicians as they counsel patients with false positive 50 g GCT screening tests.

## A-211

### Evaluation of a plasma renin mass assay as a replacement for plasma renin activity measurement.

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**Background:** Plasma renin is measured in the workup of refractory hypertension to aid in the diagnosis of primary hyperaldosteronism. The Endocrine Society (ES) recommends screening a defined subset of hypertensive patients with the aldosterone / renin ratio (ARR), with follow-up of patients with abnormal results (based on both the ratio **and** increased aldosterone concentration) with more definitive confirmatory testing. Available direct renin mass (DRM) assays present an attractive alternative to PRA for the laboratory because it is performed on an automated, random access analyzer which greatly simplifies workflow compared to the RIA or LC-MSMS based PRA assay. The manufacturer's recommendations for DRM sample handling are different than those for PRA in order to reduce prorenin conversion to immunoreactive renin. **Objective:** To verify pre-analytical sample handling conditions to allow for the measurement of PRA and DRM off of the same sample submitted for routine laboratory analysis. To establish the correlation between PRA and DRM, to evaluate the clinical utility of the ARR with the DRM assay replacing PRA, and to provide clinicians with appropriate interpretive guidelines if the DRM were to replace PRA. **Methods:** Three EDTA plasma samples were drawn from each of 20 healthy volunteer donors. One sample was frozen immediately, one refrigerated for two hours, and one left at room temperature for two hours. All sample were then analyzed by the DRM assay. For assay correlation, 256 samples submitted to the University of Michigan Hospital Special Chemistry laboratory for PRA utilizing a Diasorin RIA kit were also analyzed for DRM on the Diasorin Liason XL. Of these, 188 samples also had aldosterone orders. The ARR was calculated and compared for both the PRA and DRM assays. **Results:** By both paired t test and ANOVA, no statistically significant difference between the sample handling conditions could be demonstrated. P values were 0.903 for the t test and 0.99 for ANOVA. Comparison of the DRM (Y axis) to PRA (X axis) showed a strong linear correlation, with regression equation (Y = 9.05 X + 0.86, r = 0.9620. Comparison of ARR Mass (Y) to ARR Activity (X) showed a slightly poorer but still strong correlation, Y = 0.08 X - 0.05, r = 0.9272. Using a ARR Activity ratio of 20 and an ARR Mass ratio of 2.2 as cutoffs, 127 patients screened negative by both criteria, 41 patients screened positive by both criteria, and 20 patients disagreed with an ARR Activity ratio > 20 but an ARR Mass ratio of < 2.2. However only 4 of these 20 patients had an aldosterone > 15 ng/dL, the suggested requirement in the ES guidelines. **Conclusions:** Immediate post-draw sample handling conditions did not alter DRM as long as samples were centrifuged and frozen with 2 hours. DRM shows a strong correlation with PRA and is a promising potential alternative. Surveys of clinicians at our institution found multiple uses of PRA and ARR. Precise understanding of the relationship between DRM and PRA will be critical to ability to the education of all users for future implementation of DRM.

## A-212

### Evaluation of Analytical Performance of Capillary 2 Flex Piercing against Primus Ultra2

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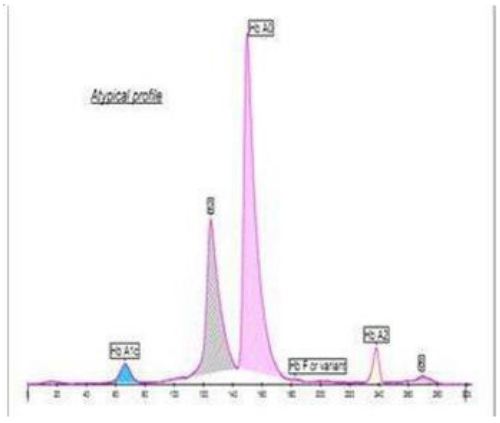
**Background:** HbA1c is an important indicator for the monitoring of glycemic levels in diabetic and prediabetic patients. It could be measured by various methods. Here we report the results of the evaluation of Capillary 2 Flex Piercing, an analyzer using capillary electrophoresis for the separation and quantification of HbA1c against Primus Ultra2,an analyzer using boronate affinity HPLC.

**Methods:** All studies were evaluated using CLSI guidelines. The precision, accuracy, linearity were evaluated according to CLSI protocols EP15-A2, EP9-A2 and EP6-A

respectively. Also we have evaluated the influence of HbE and Hb New York regarding HbA1c assays.

**Results:** Intra-tube and between-tubes CVs are respectively lower than 1.8% and 1.26%; The linearity was excellent for HbA1c values ranging from 31 mmol/mol(5.0%) to 138 mmol/mol(14.8%) (r=0.999);The results were well correlated with those obtained by the BA-HPLC method routinely used in the laboratory(Primus Ultra2):HbA1c[CapillaryS2]=0.9926*HbA1c[Primus]-0.0441(r=999); For accuracy :the bias for 39 of the 40 samples were within the range of ±6%;The analytical system were confirmed free from interference of HbE and Hb New York: the deviation of CapillaryS2 to Primus Ultra2 ranges from -0.35 to 0.34 for HbE and from -0.15 to 0.16 for Hb New York; the bias of CapillaryS2 to Primus Ultra2 ranges from -4.8% to 3.0% for HbE and from -2.9% to 3.0% for Hb New York;

**Conclusion:** This evaluation showed that the analytical performances of Capillary 2 Flex Piercing analyzer for HbA1c assay fulfilled quality criteria requested for clinical use for routine practice.




**HbA1c result of a Hb New York carrier with Sebia Capillarys 2 Flex Piercing**

## A-213

### RNase L is Involved in Glucose Homeostasis and Insulin Resistance

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**Background:** Diabetes is characterized by hyperglycemia mainly due to defect in insulin secretion and/or action. Regulation of glucose transport and use by insulin is central to the maintenance of whole-body glucose homeostasis. One of the potential mechanisms associated with insulin sensitivity is the activation of insulin receptor (IR) and subsequently transduces the signal through phosphorylation of insulin receptor substrate (IRS)1 and activation of the PI-3K/Akt pathway. In contrast, activation of the mechanistic target of rapamycin (mTOR) and ribosomal protein S6 kinase (p70S6K) inactivates the signal cascade. RNase L, an IFN-inducible enzyme, plays an important role in IFN functions against viral infection and cell proliferation. However, a direct link between RNase L and insulin sensitivity has yet to be clearly established.

**Methods:** Primary RNase L+/+ and -/- mouse embryonic fibroblasts (MEFs), hepatocytes and adipocytes were used to investigate the role of RNase L in insulin signaling and sensitivity. Cells were treated with insulin at various time points and different concentrations. Activation of the insulin signaling pathway was determined by immunoblot analyses for the protein level and phosphorylation status of these components such as IR/p-IR, IRS1/p-IRS1 and AKT/p-AKT in the presence or absence of chemical inhibitor.

**Results:** We found that RNase L plays an important role in glucose homeostasis through impacting insulin receptor (IR) which is a trans-membrane receptor activated by insulin. The phosphorylation status of IR was significantly reduced in the cells deficient RNase L. As a result, activation of IRS1, the downstream substrate of IR, and the PI3K/AKT pathway was significantly inhibited in RNase L-/- cells. Further investigation of the molecular mechanism underlying the role of RNase L in mediating the activation of IR revealed that RNase L may regulate the cleavage of the precursor of IR via the ubiquitin/ proteasome system. In addition, the level of activated S6 kinase in the mTOR pathway was also markedly elevated.

**Conclusion:** In summary, the role of RNase L in the insulin signaling pathway suggests that RNase L may be a novel target in the design of therapeutic strategies for diabetes. Treatment of this disease may be achieved through regulating the expression and activation of RNase L. In addition, RNase L may be used as a prognostic marker for diabetes as well.

## A-215

### Unexpectedly high adrenocorticotropic hormone values due to its complex formation with immunoglobulin

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**Background:** Pituitary-incidentaloma is unsuspected lesion incidentally detected on an imaging-study. Despite lacking of clinical symptoms for a specific pituitary disease, routine functional-evaluation is recommended. This condition can cause a disparity between patient's clinic and laboratory results. Here, we present a pituitary-incidentaloma case with unexpectedly-high adrenocorticotropic-hormone (ACTH) levels, subsequently confirmed as macro-ACTH. **Methods:** A 28-year-old male patient was admitted to the hospital to assess hormonal functions of a pituitary-adenoma which was detected by cranial-MRI performed for differential diagnosis of headache. Past-medical history only revealed a two-year course of anti-epileptic treatment after a head injury. There were not any stigmata on physical examination suggesting pituitary hypo-hyperfunction. Pituitary-MRI revealed a suspected pituitary-microadenoma (6x3.5 mm) at the left-posterior-segment of the gland. Results of pituitary-hormone function were shown in Table-1. Plasma-ACTH was measured by IMMULITE2000 (Siemens-Co, USA), which is a solid-phase two-site immunometric-assay including monoclonal-murine anti-ACTH and polyclonal-rabbit anti-ACTH antibodies. Serum cortisol levels were measured by DXI800 (Beckman Coulter, Co, USA), based on competitive-immunometric assay using paramagnetic particles coating with goat anti rabbit-IgG, and rabbit anti-cortisol antibodies. **Results:** Repeated measurements of ACTH were high [150, 167, 172 pg/mL (0-46)]. Cortisol was suppressed after 1mg overnight dexamethasone suppression test but ACTH not (Cortisol: 1.9, ACTH: 163). ACTH measurement interference was suspected. Plasma-ACTH was measured by electrochemiluminescent assay (ROCHE Diagnostics, USA), and found 199 pg/mL. Dilution study of patient plasma was non-linear by IMMULITE2000. HBT study (Scantibodies Laboratory, Inc., Santee, CA, USA) showed that there was no heterophilic antibody interferences. A 22.53% recovery was detected by PEG precipitation study. These results show that high ACTH levels are due to its complex formation with immunoglobulin, called macro-ACTH. **Conclusion:** Incompatible high ACTH levels can complicate to detect pituitary adenomas. Clinicians should be aware in terms of prevent unnecessary advanced investigations. Macro-ACTH could reflect pituitary adenoma's altered hormone production or could be a coincidental finding.

Hormone levels of the patient.

| Parameter | Value |
|---|---|
| ACTH (pg/mL) | 176 (0-46) |
| Cortisol (µg/dL) | 11.33 (7-23) |
| TSH (µIU/mL) | 1.4 (0.34-5.6) |
| fT3 (pmol/L) | 7.1 (3.8-6) |
| GH (ng/mL) | 0.49 |
| IGF-1 (ng/mL) | 144 (80-644) |
| FSH (mIU/mL) | 3.8 (1-19) |
| LH (mIU/mL) | 3.9 (2-9) |
| Testosterone (ng/dL) | 512 (181-772) |
| Prolactin (ng/mL) | 7.9 (2-13) |
| fT4 (pmol/L) | 7.9 (2-13) |

## A-216

### Biotin May Lead To High Free Thyroxine Levels in Some Immunoassay Methods

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**Background:** Biotin is required for carboxylases including acetyl-CoA carboxylase in cytosol and, pyruvate carboxylase, propyonyl-CoA carboxylase, methyl-crotonyl-CoA carboxylase in mitochondria; all are important for fatty acid synthesis, amino

acid metabolism, and gluconeogenesis. Biotin deficiency leads to reduced carboxylase activities, disruption of energy metabolism, and increased organic acids in urine. In brain, biotin deficiency causes lactate accumulation, then seizures and ataxia. Therefore biotin replacement is required.

**Methods:** Here we report two children taking biotin-replacement therapy with high free-throxine (FT4) and free-triiodotyronin (FT3) levels. First patient is a girl, 1y4d, with 61.93 pmol/L (7,86-14,4 pmol/L) of FT4, 14,99 pmol/L (3,8-6,0 pmol/L) of FT3 and 5.04 ug/mL( 0,38-5,33 µg/mL) of TSH levels. Her serum anti-TPO and anti-TG levels were in reference intervals. She was hospitalized with preliminary diagnosis of fatty-acid oxidation defect. She was taking 1 mg/d biotin. The second patient is a boy, 1y4m, with 21.2 pmol/L of FT4 and 1.12 µg/mL of TSH levels. His biotinidase activity was found 1.9 U/L (>3.5 U/L), so was started to use biotin with the diagnosis of partial biotinidase deficiency.

**Results:** All measurements were performed by DXI800 (Beckman Coulter, Co, USA). FT4 levels were measured by another method, ECLIA (ROCHE diagnostic, USA) and found 1.56 ng/dL and 1.48 ng/mL (1.02-1.72 ng/dL), respectively. Beckman-FT4 measurement is a two-step chemiluminescent assay using monoclonal mouse anti-T4 antibody labeled with biotin. Beckman-FT3 assay is a competitive binding immunoenzymatic assay, in which biotinylated-T3 analog is used. In both, at the end of reaction, the substrate and ALP-conjugate are added, and light is produced, that is inversely proportional with analyte-concentrations. Biotin leads to decrease of light, so FT4/FT3 levels are increased. It was confirmed by adding biotin to the sample on DXI800 (Table 1).

**Conclusion:** Clinicians should check whether the patient has received biotin-therapy in high FT4/FT3 results incompatible with the patient's clinic.

## A-217

### Evaluation of the Bio-Rad D-100™ system for the measurement of glycated hemoglobin ($HbA_{1C}$)

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**Background**: $HbA_{1C}$, the main form of glycated hemoglobin, is the gold standard for the monitoring of glycemic control in diabetic patients and has recently been recommended for the diagnosis of diabetes. $HbA_{1C}$ levels also correlate with the development of long-term complications in diabetic patients. It is therefore essential that $HbA_{1C}$ measurements be performed on robust and reliable methods. The aim of this study was to evaluate the D-100™ system (Bio-Rad Laboratories) for the accurate quantification of $HbA_{1C}$.

**Methodology**: Detection of $HbA_{1C}$ in whole blood by the D-100 system is based on ion-exchange quantitative high performance liquid chromatography (HPLC) in a 45 second separation per sample. Precision was assessed for 24 days by measuring Bio-Rad quality control (QC) materials in addition to four patient samples, in duplicate, twice daily. Linearity and accuracy was assessed using proficiency testing (PT) material from the College of American Pathologists (CAP) or Institute for Quality Management in Healthcare (IQMH). Remnant samples after routine analysis were collected and utilized for comparative testing against the Bio-Rad VARIANT™ II Turbo. Interference from known hemoglobin variants (AC, n=55; AD, n=41; AE, n=43; AS, n=37) was assessed by comparing results to those obtained by the Trinity Biotech ultra$^2$ boronate affinity HPLC at a NGSP reference laboratory. An overall test of coincidence of least-squares regression lines was used to test for statistically significant differences compared to AA samples; clinical significance was defined as a relative difference exceeding ±7% versus AA samples at HbA1c levels of 6 and 9 $\%HbA_{1C}$ based on Deming regression..

**Validation**: The Bio-Rad Lyphocheck and Liquicheck QC showed within run and total coefficient of variation (CV) of 0.8-1.0% and 0.9-1.1%, respectively. $HbA_{1C}$ levels in patient samples ranging from 4.8 $\%HbA_{1C}$ to 12.1 $\%HbA_{1C}$ showed total CVs of 0.7–0.8%. Linearity over a measuring range of 5.10-11.17 % $HbA_{1C}$ was acceptable with a slope of 0.947 and intercept of -0.06. PT sample results met CAP and IQMH criteria (allowable error of 6% and 7%, respectively). For the method comparison, samples were selected to maximally cover the measuring range of the assay, 3.5 $\%HbA_{1C}$ to 20.0 $\%HbA_{1C}$. One hundred samples were run in duplicate on the D-100 analyzer and compared to routine measurements on the Bio-Rad Variant II Turbo analyzer. Deming regression analysis showed R=0.9983, slope of 0.944 (0.937-0.952), y-intercept of 0.08 (0.03 – 0.14); the standard error of the estimate was 0.09 $\%HbA_{1C}$. Bland-Altman analysis showed a mean difference of -0.3 $\%HbA_{1C}$ (95% CI: -0.5 – 0.0 $\%HbA_{1C}$). The variant interference evaluation showed no clinically significant interferences for the four variants tested, although there were statistically significant differences for AE and AS ($p<0.05$). In addition, the D-100 Advisor software correctly provided the presumptive identification of the 176 known AS, AC, AD, and AE variants according to defined chromatographic time windows.

**Conclusions**: The Bio-Rad D-100 system is a robust, high-throughput method for the routine determination of $HbA_{1C}$ in clinical laboratories.

## A-218

### Microparticles expressing tissue factor as a marker for antithrombotic metformin effect in polycystic ovarian syndrome

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**Background:** Microparticles (MPs) are extracellular vesicles released by the cell membrane during apoptosis or cell activation and are potential mediators of several diseases, since they act as signaling mediators. These structures are increased in women with Polycystic Ovarian Syndrome (PCOS), which is related to thrombotic complications. Several patients with PCOS use metformin because of their hypoglycemic effect, but it is not clear if metformin also improves the hemostatic profile, reducing the thromboembolic risk. This study aimed to evaluate whether the microparticles expressing TF (TFMPs - an important pro-coagulant marker) are altered in PCOS women under metformin treatment. **Methods:** PCOS diagnosis was performed according to the Rotterdam criteria. We quantified the TFMPs in citrate plasma of 50 patients with PCOS - 13 of these women used metformin (850 mg 2x/day during at least 6 months) and the other 37 did not. The TFMPs were quantified in a BD LSRFortessa® flow cytometer. The presence of phosphatidylserine (marker for MPs) was determined based in the interaction with annexin V, using fluorescein isothiocyanate (FITC) conjugated monoclonal antibodies. Specific monoclonal antibodies CD142-PE to identify MPs expressing tissue factor were also applied. Trucount control tubes were included as a quality control. All data were analyzed by statistics software SPSS (13.0 version). We used the Shapiro-Wilk test to determine the normality. Considering that the distribution was parametric, test t-Student was assess in order to compare the two groups. $p<0.05$ value was considered significant

**Results:** Plasma levels of TFMPs were significantly lower in the group of patients who used metformin when compared to untreated women ($p = 0.003$). **Conclusion:** These results suggest the use of TFMPs as marker to evaluate the antithrombotic effect of metformin. This study included PCOS women, but it could be extended to other diseases associated to insulin resistance and hypercoagulability, which require metformin treatment.

## A-219

### Performance Evaluation of the VITROS® Immunodiagnostic Products Insulin Test* on the VITROS® ECi/ECiQ Immunodiagnostic Systems, VITROS® 3600 Immunodiagnostic System, and VITROS® 5600 Integrated System

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**Background:** We have developed an enhanced chemiluminescent assay for the measurement of human insulin on the VITROS® ECi/ECiQ Immunodiagnostic System, VITROS® 3600 Immunodiagnostic System, and VITROS® 5600 Integrated System. Performance verification testing was conducted to evaluate precision, Limits of Blank, Detection and Quantitation, comparison to three commercially available insulin assays and to establish a reference interval.

**Methods:** Total Within Lab Precision was evaluated per CLSI EP05-A3 by testing a 5 member panel with concentrations ranging from 5.81 to 206µIU/mL in duplicate 2 times per day for 20 nonconsecutive days. Testing spanned a total of 29 days and included 5 calibration events. Limits of Blank, Detection and Quantitation (LoB, LoD, LoQ) were evaluated per CLSI EP17-A2 by testing 10 replicates of each of 4 LoB fluids and 5 LoD/LoQ fluids 2 times per day for 5 days across 3 calibration events. A total of 130 samples that spanned the assay range were tested in the VITROS Immunodiagnostic Products Insulin Test and an aliquot was sent out for testing on 3 commercially available automated comparator methods. The sample set included random samples, fasting samples, and post meal samples collected from in house volunteer participants. Passing Bablok regression was used to compare the methods. A reference interval for 99 apparently healthy, fasting individuals was established according to CLSI EP28-A3C using a parametric analysis and log-normal transform

estimate at the 95% confidence level. All verification testing was conducted using 3 reagent lots across the VITROS ECi, VITROS 3600 and VITROS 5600 systems.

**Results:** The total within lab precision estimates ranged from 2.5% to 7.0% among the 5 panel members across the reagent lot/system combinations. The LoB is 0.033µIU/mL based on 400 determinations of four blank samples. The LoD is 0.077µIU/mL based on 500 determinations of five low-level samples. The LoQ based on 500 determinations with the five LoD pools; and a precision goal of 20% using the functional sensitivity method is 0.077µIU/mL. For the method comparison, Passing Bablok regression analysis yielded a slope of 0.79, intercept of 0.01 and Pearson Correlation Coefficient of 1.00 for comparator method 1; a slope of 1.13, intercept of 0.30 and Pearson Correlation Coefficient of 1.00 for comparator method 2; a slope of 0.86, intercept of -0.28 and Pearson Correlation Coefficient of 1.00 for comparator method 3. The reference interval for the VITROS Insulin Test was 2.30 to 26.0µIU/mL.

**Conclusion:** The performance verification data demonstrate that the VITROS® Immunodiagnostic Products Insulin Test has comparable precision, Limit of Detection/Quantitation, correlation with three commercially available methods, and a fasting reference interval consistent with comparator methods.

*Under development

## A-220

### Thyreoglobulin and anti-thyreoglobulin in the needle washout of neck lymph node biopsies suspected of metastasis of differentiated papillary thyroid cancer

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**Background:** Several studies report that detection of thyroglobulin (Tg) in fine-needle aspiration (FNA) biopsy washout fluid from lymph nodes identifies recurrences/metastases of differentiated papillary thyroid cancer (DPTC) in the neck with higher sensitivity and specificity than fine-needle aspiration cytology (FNAC). However, there are few data on the levels of anti-Tg antibodies in these washouts (TgAb-FNAB), with a restricted number of samples, which compromises the ability to draw further conclusions. **Methods:** To measure Tg-FNAB and TgAb-FNAB in washout samples from patients submitted for FNAB due to the suspected presence of metastases of DPTC in neck lymph nodes. This is a transversal study that enrolled 100 samples for determination of Tg-FNAB and TgAb-FNAB in neck aspirate of lymph nodes suspected of having metastatic disease of DPTC. The study was conducted from January to October 2016. The presence of TgAb-FNAB was analyzed in each sample by two different immunofluorimetric assays (Siemens and Roche). The cutoff value for increased Tg-FNAB was 0.1ng/dL and for increased TgAb-FNAB was 30 IU/mL and 10 IU/mL for Siemens and Roche assays, respectively. **Results:** Among the 100 samples analyzed, 55% were positive for determination of Tg-FNAB. Of these, 34.55% (19/55) were positive TgAb-FNAB using Siemens assay and 62.22% (28/45) using Roche assay. A total of 35.56% (16/45) presented a positive result in both assays and 52,7% in at least one. All samples with negative Tg also had negative TgAb-FNAB by both assays. **Conclusion:** It is still controversial whether the presence of TgAb-FNAB interfere with the assessment of Tg-FNAB. Although previous studies did not find TgAb-FNAB in lymph nodes with positive Tg- FNA, the present study detected TgAb-FNAB in more than half of the analyzed samples. Prospective studies with a larger number of patients are important to identification of a possible causal relationship between levels of TgAb-FNAB and values of Tg-FNAB in patients investigated for presence of metastases of DPTC in neck lymph nodes.

## A-221

### Automated Dispersive Pipette Extraction of Diphenylborinate Complexed Free Catecholamines and Metanephrines in Urine with LC-MS/MS Analysis

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**Background:** The catecholamines are bioamines that play an integral role as neurotransmitters in the nervous system. Screening for catecholamines and their O-methylated metabolites is an accepted approach for diagnosis of catecholamine-secreting tumors. Generally, these tumors are benign, but potent effects on the cardiovascular system caused by excess catecholamines can have potentially fatal outcomes. Correct, timely diagnosis is crucial. Unlike plasma, it's recommended both metanephrines and catecholamines be measured in urine. Urine analysis is non-invasive and exhibits sufficiently high levels of target compounds.The proposed method uses diphenylborinic acid (DPBA) as a complexing agent to stabilize and increase lipophilicity for reversed phase retention. Dispersive pipette extraction (DPX) takes place within a pipette tip, which facilitates an easily automated alternative to traditional SPE requiring less sample and solvent volume. The objective was to develop an automated sample preparation method utilizing DPBA with DPX extraction for minimized sample preparation time and high sensitivity analysis of epinephrine, norepinephrine, dopamine, metanephrine, and normetanephrine in urine with LC-MS/MS.**Methods:** A well plate with 300 uL of sample and internal standard was loaded onto a Hamilton Microlab® NIMBUS96® system. Reservoirs of DPBA solution, wash buffer, methanol, and 1M formic acid were also added to the system deck. The system added 600 uL of complexing agent to the urine sample well plate, 500 uL of wash buffer to a second "wash" well plate, 270 uL of 1 M formic acid and 30 uL of methanol to the third "elution" well plate. The system picks up 1 mL DPX RP (reverse phase) tips. After conditioning, the tips aspirated and dispensed the sample solution four times to bind the complexed analytes. After washing with wash buffer, analytes were eluted with 1M formic acid/10% methanol solution. The acidic solution reverses diphenylborinate complexes. Low methanol content aids elution, maximizes selectivity and allows direct injection. The "elution" well plate was placed into the autosampler. This automated process takes less than 15 min to complete. **Results:** Calibrations from 0.1/0.5-1000 ng/mL resulted in average coefficients of determination ($R^2$) values over 0.998 for all analytes. The limits of detection (LOD) and quantitation (LOQ) were calculated using the average slope and y-intercept standard deviations which resulted in LODs below 0.25 ng/mL and LOQs below 0.7 ng/mL. The method accuracy was determined via quantitation of two levels of quality control samples and each average analyte concentration fell within manufacturer's expected concentration ranges. The average within-run precision was highest at 6% CV for level 1 epinephrine, and between-run precision was highest at 7% CV for level 2 metanephrine. Matrix effects were low with a range of ion suppression from 1-14% except for norepinephrine with ion suppression at 39%. Extraction efficiencies were higher than 96% for all analytes except dopamine, which resulted in 81% efficiency. **Conclusion:** The method reported herein achieves accurate, sensitive analysis of free catecholamines and metanephrines in urine. This method is an excellent alternative to previously published methods, with advantages of ease of implementation, robustness, high sensitivity, and high throughput with 96 samples extracted in less than 15 min.

## A-222

### Low Serum Alkaline Phosphatase Activity in a Teenage Girl

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**Background:** Hypophosphatasia (HPP) is a rare and genetic disorder that affects bone mineralization. There are currently six forms of HPP that range in age of onset and severity: perinatal (lethal), perinatal benign, infantile, childhood, adult and odontohypophosphatasia. Perinatal HPP is the most severe and results in death in utero, whereas adult HPP is milder and presents with pain and osteomalacia. HPP is caused by mutations of the *ALPL* gene encoding tissue-non-specific alkaline phosphatase. One of the cardinal features of HPP is a low serum alkaline phosphatase (ALP) activity. Recognition of HPP and differentiation from other causes of ALP activity is required for proper diagnosis and treatment. Here we present a clinical case of a 17-year-old girl who presented with fatigue at her annual medical exam. Results from an external laboratory showed low daytime cortisol concentration. She was referred to endocrinology for chronic fatigue and possible adrenal insufficiency per her father's request. Family history was notable for hypothyroidism and chronic fatigue syndrome. Her medical history was notable for the presence of anti-TPO antibodies below the diagnostic threshold and a normal TSH. Of note she was taking folate, vitamin B12, and multi-vitamins. During her initial workup laboratory results were notable for low ALP activity (24 units/L; reference interval 55-140 unit/L). Low ALP activity can be due to Wilson's disease, hypophosphatasia, pernicious anemia and severe hypothyroidism. Magnesium and zinc deficiencies were once sources of falsely low ALP measurements but current assay formulations incorporate magnesium and zinc to circumvent this issue.

**Methods: :** In order to differentiate the cause of her low serum ALP activity, new samples were collected and ALP activity repeated. In addition ALP-isoenzyme analysis, sequencing of the ATP7B gene, ceruloplasmin, vitamin B6 (P5P) and urine phosphoethanolamine quantification were also performed.

**Results:** The repeat analysis of the patient's serum ALP activity was 23 units/L. ALP-isoenzyme analysis was not possible due to insufficient ALP activity. Initial results showed elevated concentration of vitamin B12 and normal thyroid function,

eliminating pernicious anemia and hypothyroidism from the differential. The patient's ceruloplasmin concentration was quantified as 16.5 mg/dL (reference interval 16-45 mg/dL) prompting further evaluation for Wilson's disease via sequencing of the *ATP7B* gene which did not reveal any deleterious mutations. Vitamin B6 and PEA concentrations were 95 µg/L; 5-50 µg/L) and (80 mmol/mg Cr; <88 mmol/mg Cr), respectively, values consistent with hypophosphatasia.

**Conclusion:** Due to the absence of bone abnormalities and impaired growth, HPP was initially considered unlikely; however, the combination of low ALP activity and high vitamin B6 and PEA are consistent with a diagnosis of hypophosphatasia. Her clinical features suggest a mild form of childhood or adult HPP. This case was complicated by ceruloplasmin concentration at the lower limit of the reference interval. The diagnosis was also complicated by a later admission that the patient was receiving cortisol from her father in order to treat her fatigue. The family was advised to stop giving exogenous cortisol and the patient successfully tapered off without any signs of an acute adrenal crisis. The family declined genetic testing of the *ALPL* gene.

## A-223

### Prolactin heterogeneity in inferior petrosal sinus samples

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Background:

Measuring prolactin levels in inferior petrosal samples may aid in differentiating between pituitary and ectopic ACTH dependent Cushing's. However, circulating peripheral prolactin is known to exhibit molecular heterogeneity but its presence in inferior petrosal (IP) blood is unknown. This project examined the presence of macroprolactin as well as glycosylated forms in inferior petrosal blood draining the pituitary.

Methods:

Twenty one matched samples from peripheral blood, left inferior petrosal, and right inferior petrosal veins were obtained from seven patients being investigated for ACTH-dependent Cushing's.

Prolactin heterogeneity was examined as follows; the presence of macroprolactin variant was investigated using polyethylene glycol (PEG) precipitation (following incubation of 100 ul sample for 20 minutes at room temperature with an equal volume of 25% (v/v) PEG and centrifugation at 1400 xg for 5 minuets), and by immunoadsorption using protein-G suspension (Thermo Scientific, MA, USA). 100 ul sample was incubated with 50 ul Protein-G suspension with agitation for 60 minutes at room temperature before separation on a magnetic rack and elution using 0.1M glycine buffer (pH 2.0) and adjusting pH to 7.4. The presence of glycosylated variants was examined using Concanavalin-A lectin columns (GE Healthcare, USA). 100 ul sample was applied to 1 mL column and bound prolactin was eluted using 0.5M methyl-alpha-D-glucopyranoside. Prolactin levels prior to and following the above treatment protocols were measured using ELISA (Calbiotech, CA, USA) according to manufacturer's instructions.

Results:

Peripheral blood prolactin ranged from 7.9 to 83.5 ng/mL, while left IP sample prolactin levels ranged from 24.1 to 189.0 ng/mL, and right IP sample prolactin levels were from 87.3 to 524.1 ng/mL. None of the samples exhibited macroprolactin, as percentage PEG precipitated prolactin was <40% for all. Similarly, none of the samples showed immunoglobulin bound prolactin evident by all percentage protein-G bound prolactin of <2.6%. However significant glycosylated prolactin variant was present. Percentage of glycosylated prolactin variant ranged from 11.2 to 45.3%.

Conclusion:

The presence of molecular variants in petrosal sinus blood was not previously known. This study showed that although macroprolactin variant was not present in neither petrosal nor peripheral blood from patient being investigated for ACTH dependent Cushing's, a significant proportion of the circulating prolactin was glycosylated. There was no significant difference in the proportion of glycosylated prolactin between peripheral, left or right IP veins. The pathogenesis as well as the impact of prolactin glycosylation on its diagnostic utility needs to be investigated.