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 Tuesday, August 2, 2016
 

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

Immunology

A-303

**Molecular Allergology - A 'MUST' Clinical Laboratory Service for Correct Allergy Diagnosis and Targeted Patient Management**
V. M. Lo. *Hong Kong Sanatorium and Hospital, Happy Valley, Hong Kong*

**Background:** While measuring allergen specific immunoglobulin E (IgE) can help allergy diagnosis, it does not differentiate genuine allergens from cross-reacting species and cannot distinguish heat stable from labile proteins. Thus an incomplete assessment of an allergic patient makes clinical management suboptimal.

**Method:** In October 2012 our laboratory was the first in a Hong Kong private hospital to provide a molecular allergology service using Immunocap 100, Phadia (Pharmacia Diagnostics), to measure IgE to five peanut components, namely rArah1, rArah2, rArah3, rArah8, and rArah9. rArah1, rArah2, rArah3 and rArah9 are heat stable proteins, while rArah8 is heat labile and cross reacting component. Cases were referred after screening positive for allergen specific IgE by blood and/or skin prick test, or if there was a strong history of symptoms elicited by peanut.

**Results:** Fifty-eight out of 795 peanut allergic cases, 7.3%, were received, 38 were boys and 20 were girls, aged 1-10 years. Patients were categorized into three groups according to their responses to each of the allergen component. Group 1 was composed of fifty-two children, they were genuine peanut allergic with IgE level at RAST score 3 - 6 to either one or all of rArah1, rArah2, rArah3 and rArah9. Group 2 consisted of four children, they showed response to heat-labile cross-reacting component, rArah8, only with IgE level at RAST score 2 - 3. Group 3 had two children demonstrated no response to peanut specific markers or cross-reacting components available for testing. Different group associated with different targeted treatment regime. Group 1 children with moderate to high levels of IgE to the respective heat stable storage proteins (rArah1-3 and 9) were advised to avoid peanut entirely. As the risk of anaphylaxis is small in group 2 children with only IgE to the heat labile PR-10 protein rArah8, eating peanuts is allowed but only when they are well cooked and after a supervised oral challenge in the Allergy Centre has shown that this is safe. Since peanut allergic is not ruled out, group 3 children were advised to avoid peanut entirely.

**Conclusions:** Precise identification of sensitization to specific allergen components by molecular allergology can facilitate targeted patient management.

A-304

**Urinary interleukin(IL)-18 as an early predictive biomarker of subclinical proximal tubular dysfunction in HIV-infected patients exposed to Tenofovir.**
C. G. Orluwene<sup>1</sup>, N. Deebii<sup>2</sup>, E. P. Odum<sup>1</sup>. <sup>1</sup>University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria, <sup>2</sup>University of Port Harcourt, Port Harcourt, Nigeria**BACKGROUND**

Proximal tubular dysfunction (PTD) is a frequent complication of HIV-infected patients and lack of early biomarkers for PTD has impaired our ability to intervene in a timely manner considering the increasing number of HIV-infected patients in Nigeria.

**AIM**

In this present study, we tested if interleukin- 18 (IL-18) is a predictive biomarker for PTD in HIV-infected patients on tenofovir (TDF).

**SUBJECTS AND METHOD**

HIV-infected patients on tenofovir (TDF) and Non-tenofovir (N-TDF) antiretroviral therapy were recruited for this study, taking as control HIV treatment-naïve patients. Exclusion criteria included pre-existing renal insufficiency and nephrotoxin use. Serial urine samples were analyzed by enzyme-linked immunosorbent assay for IL-18 in 254 HIV- infected patients at three different points (at baseline, at 4 weeks and at 12 weeks of follow-up).

**RESULT**

Using eGFR values, marked decrease in kidney function was detected only at 12 weeks in the TDF regime group (p=0.003) as compared to other study groups. In contrast, urine IL-18 increased at a much early time (at 4 weeks) particularly in the TDF regime group (p=0.000) followed by the naïve group (p=0.02) and continued to increase up to 12 weeks of follow up. This marked elevation is progressive.

**CONCLUSION**

Our results indicate that IL-18 is an early, predictive biomarker of PTD and that this biomarker may allow for the reliable early diagnosis of PTD at all times in HIV-infected patients on TDF at risk of proximal tubular dysfunction, much before the rise in serum creatinine.

**Keywords:** Urinary-interleukin-18; Biomarker; HIV-infected-patients; Proximal-tubular-dysfunction.

A-305

**Evaluation of the AdvanSure Alloscreen max panel for detecting multiple allergen-specific IgEs in Korean allergic patients: Comparison of AdvanSure Alloscreen max panel, Polycheck Allergy, and ImmunoCAP**
S. Chae, J. Chung, H. Huh. *Dongguk Univ Medical Center, Goyang, Korea, Republic of*

**Background:** Allergen-specific IgE blood tests for diagnosing allergy are less affected by administration of antihistamines than skin prick and display numerical results that may be important for consecutive monitoring. The ImmunoCAP (Phadia, Uppsala, Sweden) is accepted as a standard allergen-specific IgE measurement, but it is expensive and detects only one allergen-specific IgE. In contrast, multiallergen IgE screening assays are more cost-effective because they detect IgE antibodies to common allergens in a single test. A new generation AdvanSure Alloscreen max panel (LG Life Science, Seoul, Korea) has been introduced as a multiallergen IgE screening assay. This study evaluated performance of the AdvanSure Alloscreen max panel compared with that of Polycheck Allergy (Biocheck Co, Munster, Germany) and ImmunoCAP in Korean patients with allergy.

**Methods:** A total of 185 patients who showed class 1 or higher results to one or more allergens on the Polycheck Allergy assay and who were diagnosed with an allergic disease were included. The AdvanSure Alloscreen max panel was performed on 185 serum samples. A total of 58 allergens with similar reagent codes in the AdvanSure Alloscreen max panel and Polycheck Allergy were selected to compare matched results, and 7,151 matched result sets were obtained. Among the 588 matched result sets with discrepancies (class 0 or 1 in one assay and class  $\geq 2$  in another assay), 566 available cases were tested with ImmunoCAP.

**Results:** Positive rates to allergens varied between the AdvanSure Alloscreen max panel and Polycheck Allergy assays. The 0-3-year age group showed high positive rates for milk, egg white, peanut, cheddar cheese, and house dust on both assays. The  $\geq 3$  year age group showed the highest positive rates for Dermatophagoides farina, D. pteronyssinus, and house dust. The AdvanSure Alloscreen max panel showed higher positive rates than those of Polycheck Allergy for those three allergens. Agreement rates (kappa values) between AdvanSure Alloscreen max panel and Polycheck Allergy for each of the 58 allergens were 53.5-98.4% (-0.12-0.85). The overall agreement rate (kappa value) between the two assays was 91.8% (0.53). Fifteen allergens had Kappa values  $> 0.6$ . The overall agreement rate (kappa value) between the AdvanSure Alloscreen, and Polycheck Allergy assays compared with ImmunoCAP were 64.5% (0.29), and 35.5% (-0.3), respectively, when the 566 matched result sets with discrepant results were analyzed by ImmunoCAP. Agreement between the AdvanSure Alloscreen max panel and ImmunoCAP was better than that between Polycheck Allergy and ImmunoCAP for 31 of 51 allergens.

**Conclusion:** AdvanSure Alloscreen max panel is a very convenient and fully automated test requiring only a 100  $\mu$ l blood sample to simultaneously measure up to 92 allergens. The AdvanSure Alloscreen max panel showed better agreement with ImmunoCAP than Polycheck Allergy. Therefore, the AdvanSure Alloscreen max panel would be a useful screening tool for detecting allergen-specific IgE on multiple allergens.

## A-306

**The associations of *TLR7* polymorphisms in autoimmune thyroid diseases**

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**Background:**

Graves' disease (GD) and Hashimoto's disease (HD) are autoimmune thyroid diseases (AITDs), and the prognosis of AITD is different from each patient. Toll-like receptors (TLRs), which play a key role in innate responses, have been identified as pattern-recognition receptors and trigger activation of signaling pathways to produce proinflammatory cytokines. It is possible that a substance coming from self becomes the endogenous ligand and causes chronic inflammation such as the autoimmune disease by TLR. Therefore, TLR7 which recognize single-stranded RNA (ssRNA) are possibly associated with the pathogenesis of autoimmune diseases. To clarify the association between TLR7 and AITD, we genotyped *TLR7* rs179009 C/T, rs179010 C/T, rs179019 A/C and rs3853839 G/C polymorphisms.

**Methods:**

Among GD patients who had a clinical history of thyrotoxicosis with positive for anti-thyrotrophin receptor antibody (TRAb), we screened patients who had been treated methimazole for at least 5 years and were still positive for TRAb (intractable GD) and patients with GD who had maintained a euthyroid state and were negative for TRAb for more than 2 years without medication (GD in remission) and patients who could not be categorized to intractable GD or GD in remission groups at the time of analysis. Among HD patients, who were positive for anti-thyroid microsomal antibody (TgAb) and/or anti-microsomal antibody (McAb), we genotyped patients who developed moderate to severe hypothyroidism before 50 years of age and were treated daily with thyroxine (severe HD), and untreated euthyroid patients who were over 50 years of age (mild HD) and patients who could not be categorized to severe HD or mild HD groups at the time of analysis. Healthy volunteers were euthyroid and negative for thyroid autoantibodies. We genotyped *TLR7* rs179009 C/T, rs179010 C/T and rs3853839 G/C polymorphisms used by PCR-RFLP method and genotyped rs179019A/C polymorphism used by Taqman PCR method in AITD and healthy control subjects.

**Results:**

Because *TLR7* gene is located in X chromosome, we analyzed the genotype and alleles of each polymorphism by sex.

1. In male, rs3853839 G allele was significantly more frequent in GD, AITD and intractable GD than that in control subjects ( $P=0.0062$ ,  $0.0232$ , and  $0.0173$  respectively).
2. In female, the rs179009 TT genotype was significantly less frequent in HD patients than that in control subjects ( $P=0.0494$ ). In male, we could not find any differences among each group.
3. In female, rs179010 CT genotype was significantly less frequent in severe HD than that in control subjects ( $P=0.0479$ ).
4. Both in male and female, we found no associations between rs179019 polymorphism and the pathogenesis of AITD.

**Conclusion:**

*TLR7* rs3853839 polymorphism was associated with the pathogenesis of GD, and *TLR7* rs179009 and rs179010 polymorphisms were associated with that of HD.

## A-308

**Natural antibody assays and Immune Status Measurement**

D. Sims, D. Taylor, A. Cook, A. Parker, S. Harding, G. Wallis. *The Binding Site Group Ltd, Birmingham, United Kingdom*

**Background:** Naturally occurring common-antigen antibodies (CaAb) are raised in response to infection or environmental exposure. Antibody deficiency disorders represent the most common type of immunodeficiency disease. Measurement of antibody binding to common exposure antigens could be used as surrogate markers of immune status. **Aim:** Determine whether CaAb activities could be used as markers of antibody deficiency.

**Methods:** An enzyme immunoassay (EIA) to detect anti-IgG and anti-IgM CaAb against bacterial (Pneumococcal C-polysaccharide and Tetanus toxoid) fungal (yeast  $\beta$ -1-3 glucan) and viral (Cytomegalovirus, CMV and Epstein-Barr virus) antigens were

developed and optimised. Serum samples from primary (primary antibody deficiency (PAD; n=20) and common variable immunodeficiency (CVID; n=20) and patients with hypogammaglobinaemia (multiple myeloma; MM, n=20) patients were analysed and compared to healthy controls (n=53). CaAb IgG was also monitored in healthy controls (n=7) for 0, 7, 14, 21, 28, 90, 180, and 360 days. Total serum IgG and IgM were measured by turbidimetric immunoassay. IgG CaAb subclass compositions were determined using subclass-specific titre matched antisera.

**Results:** IgG and IgM CaAb levels were detected in healthy adult controls for all 5 specificities using a single sample dilution (1/100). The intra-assay precision ranged from 1.8 to 4.5 %CV, and 0.73 to 4.9 % for inter-assay. There was a variable and weak correlation between the different IgG specificities (Spearman's Rho -0.302,  $p=0.0278$  to Rho 0.525,  $p<0.001$ ). Whereas, the IgM values were more closely correlated (Rho 0.66 to 0.84,  $p<0.001$ ). IgG subclass analysis showed an IgG2 bias (>76% of total IgG) towards polysaccharide antigens. Correlation between total serum IgG and IgG CaAbs were weak (Rho -0.075 to 0.457), but somewhat higher (0.602 to 0.775) for total IgM. CaAb IgG levels were remarkably stable in healthy controls for up to one year. Although the titre of each CaAb was specific to that individual the comparable order of the 5 different antigens was maintained throughout the year. 9/10 CaAb IgG and IgM titres were significantly reduced in the PAD and CVID populations compared to controls (Mann-Whitney U, all  $p<0.02$ ). Within the MM population IgG and IgM titres against the bacterial and fungal antigens was reduced compared to the control ( $P<0.003$ ). However, there was no reduction for IgG and IgM CaAbs against viral antigens. Interestingly CMV IgG levels were not suppressed in any of the immunodeficiency populations. Correlations between CaAbs and total serum immunoglobulins in immunodeficient patients were moderate for IgM (Rho 0.174 to 0.627) but weak for IgG (-0.61 to 0.03).

**Conclusion:** We have developed a multiple EIA to simultaneously measure naturally occurring antibody IgG and IgM levels to some common microbial antigens. Our results indicate that measurement of these natural antibodies can differentiate between a normal and suppressed humoral immune system and are relatively independent of total immunoglobulin concentration.

## A-309

**A new improved method to Human leukocyte antigen (HLA-B27) genotyping**

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Human leukocyte antigens (HLA) are the products of genes of the major histocompatibility complex. Some of these antigens are related to the presence of certain diseases. HLA-B27 allele is associated with ankylosing spondylitis (relative risk of 95%). The HLA-B27 is present in over 90% of caucasian individuals affected by this disease and can predict risk of transmission to their offspring. Increased incidence of HLA-B27 has been reported in Reiter's syndrome, uveitis, psoriatic arthritis and reactive arthritis. This antigen is not a disease marker, since it is present in approximately 10% of normal individuals. The result should be associated with the clinical and radiological findings suggestive of these diseases. Several methods have been developed for identification of HLA-B27. Initially, flow cytometry was used, but nowadays, due to their specificity and speed, molecular methods are used preferably. Among the main molecular methods disclosed for HLA-B27 genotyping are SSP-PCR, RFLP and PCR in real time. Several advantages are presented in these methods, as reduction the execution time of 5 hours in the PCR-SSP to 2 hours only in the real time, specificity of PCR primers and low cost. This work present a method developed in-house for the detection of HLA-B27 allele which combines PCR facilities in real time, as speed and sensitivity, with improvements over last published methods. The study has approved by ethics committee and the informed consent has dispensed because the type of sampling. A total of 186 samples that were requested for HLA-B27 typing were included with 139 negatives and 47 positives. The reaction was performed in multiplex containing the primers for detection of HLA-B27 and STR marker TPOX (AATG) as the internal control. The use of this marker as an internal control (IC) proved to be valid because its thermodynamic properties that increase the distance between the target and IC melting curves, making the test more reliable. HLA-B27 alleles and IC were identified with peaks at  $87.32 \pm 0.28^\circ\text{C}$  and  $76.34 \pm 0.31^\circ\text{C}$ , respectively. Furthermore, the modifications on the extraction method using FTA Card® provided improvements, making the process faster. One hundred and eighty six samples genotyped previously by PCR allele-specific standard method (Steffens-Nakken, 1995) were assessed by the new method, and the concordance between the two methods was 100%. We conclude that the method developed was more efficient for genotyping HLA-B27.

## A-310

**Evaluation of the ARCHITECT B•R•A•H•M•S PCT (Procalcitonin) Assay**

J. Waterston<sup>1</sup>, E. P. Chang<sup>1</sup>, M. Dombalagian<sup>1</sup>, K. Messick<sup>1</sup>, W. Messick<sup>1</sup>, L. C. Seaver<sup>2</sup>, J. Shipman<sup>1</sup>, B. Thomas<sup>3</sup>, X. Yue<sup>2</sup>. <sup>1</sup>Thermo Fisher Scientific, Middletown, VA, <sup>2</sup>Abbott Laboratories, Abbott Park, IL, <sup>3</sup>Thermo Fisher Scientific, Hennigsdorf, Germany

**Background:** Our objective is to develop and evaluate a procalcitonin (PCT) method for the ARCHITECT *i* platform family of instruments.

Procalcitonin (PCT) is a 116 amino acid protein prohormone of calcitonin. Under normal conditions intact PCT is not secreted from the thyroid and levels in circulation are very low (< 0.5 ng/mL). Circulating PCT concentrations may increase up to several hundred ng/mL in severe sepsis and septic shock. After successful treatment intervention, PCT values decrease, indicating a positive prognosis. Persistent high or further increasing levels of PCT are indicators for poor prognosis. PCT can be a useful test for diagnosis and prognosis of bacterial infection and is usually ordered along with other tests to help detect or rule out sepsis, bacterial meningitis, or bacterial pneumonia in those that are seriously ill.

**Methods:** The ARCHITECT B•R•A•H•M•S PCT assay is a fully automated two-step chemiluminescent microparticle immunoassay (CMIA) to determine the presence of procalcitonin in human plasma and serum. The method features a highly specific anti-h-Calcitonin rat MAB coupled to paramagnetic particles and serves to capture the PCT from the specimen. After incubation and wash, the 'sandwich' complexes with anti-h-Katacalcin mouse MAB conjugated with an acridinium-derivative. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of PCT in the sample and the RLUs detected by the ARCHITECT *i* System optics.

**Results:** The sensitivity, imprecision, and linearity estimates utilized procedural guidance from the Clinical and Laboratory Standards Institute (CLSI). The measuring range extends from 0.02 to 100.0 ng/mL. The limit of blank (LoB) and limit of detection (LoD) are estimated at 0.001 and 0.005 ng/mL, respectively. The limit of quantitation (LoQ) defined by the lowest concentration where imprecision is less than or equal to 20% is <0.02ng/mL. Dilutions of disease state plasma demonstrated linearity from 0.02 to 115 ng/mL. The total within-lab 20-day imprecision is <5% for patient panels from 0.04 to 90 ng/mL. The auto-dilution protocol extends the upper measuring range from 100 to 1000 ng/mL.

In a reference range study (n=300) with half males and half females, the 97.5<sup>th</sup> percentile was 0.07 ng/mL. The 90%CI range of the 97.5<sup>th</sup> percentile was from 0.05 to 0.06 for females and from 0.08 to 0.09 ng/mL for males. The method was compared to B•R•A•H•M•S PCT sensitive Kryptor with n=107 plasma specimens both fresh and frozen with concentrations from 0.01 to 59.13 ng/mL. The Deming slope to B•R•A•H•M•S PCT sensitive Kryptor was 1.03 with a correlation coefficient of 0.99.

**Conclusion:** The ARCHITECT B•R•A•H•M•S PCT assay has low imprecision, a limit of quantitation below the typical normal range of PCT, good linearity, and compares well with the B•R•A•H•M•S PCT sensitive Kryptor assay.

## A-311

**Capillary Electrophoresis Quantitation of Clonal Protein in the Gamma Zone**

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Clonal immunoglobulins (Ig) are produced by benign and malignant disorders, mostly in the electrophoresis (ELP) gamma zone as monoclonal peaks. Accurate quantitation of clonal protein is needed for diagnosis, for gauging progression of clonal disorders, and for measuring the effectiveness of malignant disease treatment. If clonal protein is a large fraction of gamma zone protein, an accurate measure is needed to calculate the non-clonal, healthy Ig by subtraction, and to warn of a high risk of bacterial infection if the healthy Ig drops below 500 mg/dL. The most common methods of estimating clonal protein are manual, graphic methods that show significant variation among users. **OBJECTIVE:** To evaluate two automated procedures developed for estimating gamma zone monoclonal protein. **METHOD:** A common manual method for peak sizing involves "skimming" - creation of a straight baseline connecting the edges of the peak, and integration of the area between the baseline and the peak. In the automated skimming method, the peak edges are found by a curve analysis algorithm, and a third degree polynomial creates the connecting baseline. The second automated method involves fitting a clonal peak to a simulated peak and calculation of the area of the simulated peak. Most clonal peaks are similar to Gauss error probability curves. A Gaussian curve fit is used by the method reported here. The analysis uses second

derivatives of points at the top of a clonal peak for fitting to a Gauss error curve, and an integral of the fitted curve. To compare the methods, ELP data from three clinical specimens with low, normal and high levels of gamma zone polyclonal Ig were downloaded from a CapillaryS(tm) analyzer. Monoclonal similar Gaussian peaks of 62, 125, 250, 500 and 1000 mg were added to the sides, shoulders or top of the gamma zones, and recovery of the added peaks was measured. **RESULTS:** The peak finding algorithm found 65 of the 75 peaks. It found all of the peaks on the low Ig curves, but did not find some of the smaller peaks on the steeper sides of the normal and high Ig curves. Recovery of the added peaks by the two methods was similar. Both methods handled peak distortion well. The Gaussian fit method's average recovery was +28 mg, and the skim method's average recovery was -18 mg. The Gaussian fit method performed better near the beta-gamma boundary if the beta-2 peak overlapped the synthetic peak, but worse at the top of the gamma curve due to positive covariance of the gamma curve and the Gaussian peak. **CONCLUSION:** In the absence of a gold standard for clonal peaks, simulated peaks of known size are excellent alternatives for method validation. The skimming method is more robust - it adjusts better to asymmetric peaks - but the Gaussian fit method is simple and is a good estimator of symmetrical peak size. Automated peak skimming is now used routinely for our ELP interpretations. Graphic illustrations are part of this study.

## A-312

**Diagnostic Performances of Cell Bound Complement Activation Products Stratified by ANA Titers in Systemic Lupus Erythematosus**

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**Background:** Abnormal complement activation and elevated anti-nuclear antibody (ANA) titers are well-documented features of Systemic Lupus Erythematosus (SLE). Clinical studies have demonstrated that complement C4d deposited on erythrocytes (EC4d) and B-cells (BC4d) are sensitive and specific biomarkers for SLE. We sought to establish the performance characteristics of these biomarkers in the context of stratified ANA titers.

**Methods:** Anticoagulated blood was collected from 462 subjects with SLE (91% females, mean age 40 years) and 436 subjects with other rheumatic diseases (non SLE: 86% females, mean age 54 years). All subjects provided informed consent. EC4d and BC4d expression was determined by fluorescent activated cell sorting (FACS) analysis and reported as net mean fluorescent intensity (MFI). ANA was measured by ELISA (Quanta Lite<sup>®</sup>, INOVA Diagnostics, San Diego, CA) and titers were categorized as negative (<20 U), positive (20-59 U), or strongly positive (≥60 U). Positive CB-CAPs consisted of either positive EC4d (>14 Net MFI) or positive BC4d (>60 net MFI). Sensitivity, specificity, likelihood ratios (LR), and Diagnostic Odds Ratio (DOR) were calculated.

**Results:** Positive ANA (≥20 U) was 88% sensitive and 55% specific in distinguishing SLE from the non-SLE group. Strong positive ANA (≥60 U) was observed in 69% SLE and 22% subjects with diseases other than SLE. Positive CB-CAPs was 62% sensitive and 88% specific in distinguishing SLE from the non-SLE group (positive LR=5.1; negative LR=0.43). As presented in the Table, the performance characteristics of CB-CAPs were higher among subjects with elevated ANA, with DOR increasing from 2.6 (ANA negative) to 12.6 (strong ANA).

**Conclusion:** CB-CAPs levels have value in diagnosing SLE and can be particularly helpful in differentiating SLE from non-SLE in the context of high ANA titers.

Performance Characteristics of CB-CAPs Stratified by ANA Titers					
ANA Status	Sensitivity	Specificity	LR + CI 95%	LR - CI 95%	DOR
Negative (<20 U)	20% (11/55)	91% (220/241)	2.3 (1.2-4.5)	0.88 (0.76-1.0)	2.6
Positive (20-59 U)	47% (42/90)	86% (84/98)	3.3 (1.9-5.6)	0.62 (0.50-0.77)	5.2
Strong Positive (≥60 U)	74% (235/317)	81% (79/97)	4.0 (2.6-6.1)	0.32 (0.26-0.39)	12.6

## A-314

**Screening Pharmacologically Active Compounds Against TSG-6, A Therapeutic Target for The Treatment of Asthma**

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**Background:** Asthma is a disorder of the respiratory system that inflames and narrows the airways. One of the key factors driving inflammation in asthma is



the increased leukocyte infiltration into the airway wall. These cells are trapped in a hyaluronan (HA)-rich extracellular matrix produced by smooth muscle cells surrounding airway vasculature and bronchi. This 'sticky' form of HA is the result of a unique protein modification. The enzyme "tumor-necrosis-factor-stimulated-gene-6" (TSG-6) transfers the heavy chains (HCs) from inter- $\alpha$ -inhibitor (I $\alpha$ I) to form the pathologic variant of HA known as the HC-HA matrix. Previously our group showed that TSG-6 not only possesses the enzymatic ability to covalently bind HC to HA, it also significantly stimulates HA synthesis by airway smooth muscle cells (ASMCs) when they are challenged by a viral mimetic. Moreover, we found that TSG-6 knockout mice develop a markedly milder form of asthma, including less HA, reduced leukocyte infiltration, and lower airway hyper-responsiveness. These findings indicate that TSG-6 has a vital role in asthma pathobiology. Therefore, we proposed to lower the levels of HC-HA crosslinking by inhibiting the action of TSG-6. **Methods:** Accordingly, we have screened a total of 7927 compounds from the Molecular Screening Core at the Cleveland Clinic using a TSG-6 activity assay. Briefly, the cross-linking chemistry of carbodiimide is used to covalently attach HA to amine groups on NH-Covalink plates. 40  $\mu$ L of recombinant TSG-6 solution (12.5  $\mu$ L; 0.016  $\mu$ g/ $\mu$ L in 5 mL PBS with Mg<sup>2+</sup>, pH 7.4) is incubated with each compound (10  $\mu$ L; 0.04 mM) for 30 min at 25°C. Then the mixture (50  $\mu$ L) is transferred into the wells that already contain 50  $\mu$ L of PBS having 4% human serum, a source of the HC donor I $\alpha$ I, followed by incubation at 37°C for 2 hours. The relative amounts of HCs transferred to HA on the plates were detected using a polyclonal antibody that binds to the HCs. An infrared secondary antibody is then used as a reporter of HC transfer. The 96-well plates were scanned on a LI-COR CLX Odyssey infrared scanner, and the relative amount of HCs per well were determined by densitometry. **Results:** Our initial results show 43 small molecule inhibitors (antagonists) against TSG-6. The percent decrease of TSG-6 activity ranged between 67% and 408%. Currently we are in the process of *in vitro* validation of 3 of the highest hit compounds. **Conclusion:** We identified 43 small molecules that inhibit HC transfer to HA via TSG-6 by screening chemical libraries in a TSG-6 activity assay.

**A-315**

**Diagnostic challenges in the study of patients with monoclonal proteins**

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**Background:** The detection of a monoclonal protein is fundamental in the diagnosis of patients with monoclonal gammopathies such as Multiple Myeloma, Primary Amyloidosis and light chain deposition disease. When the monoclonal protein is presented in low concentrations, it may be difficult to detect using conventional methods based on electrophoretic techniques like serum protein electrophoresis (SPE) and serum immunofixation (IFE). However, the quantification of serum free light chains (sFLC) is more sensitive than conventional methods. A quick and easy algorithm based in the combination of sFLC and SPE presents a high sensitivity in the diagnostic study of monoclonal gammopathies. We report our experience with six patients where the monoclonal protein was either undetectable or barely detectable by conventional methods.

**Methods:** study of six patients with suspect of monoclonal gammopathies where the algorithm (sFLC+SPE) was applied. Serum FLC were quantified by the assay Freelite (The Binding Site) and SPE were performed on Capillarys 2 (Sebia).

**Results:** The results are shown in the table.

Case	Clinical findings	Algorithm (sFLC+SPE)	Diagnosis
Man, 68 years	Macrocytic anemia (9.0 g/dl hemoglobin), rouleaux formation of erythrocytes, discrete pancytopenia.	Small peak in SPE (0.10 g/dL), sFLC ratio very abnormal (kappa=14450 mg/L, lambda=4.9 mg/L, ratio=2949) and immunoparesis	Light Chain Kappa Multiple Myeloma Stage 3 ISS
Female, 75 years	Acute kidney injury, edema and proteinuria	SPE negative, sFLC ratio very abnormal (kappa=17.7 mg/L, lambda=1800 mg/L, ratio=0.009) and immunoparesis	Primary Amyloidosis
Male, 57 years	Intense back pain	SPE negative, altered sFLC ratio (kappa=31.6 mg/L, lambda=15.4 mg/L, ratio=2.05)	Non Secretory Multiple Myeloma Stage 1 ISS
Male, 47 years	Pathological fracture	SPE negative, altered sFLC ratio (kappa=148 mg/L, lambda=5.6 mg/L, ratio=26.3)	Light Chain Kappa Multiple Myeloma Stage 1 ISS
Female, 82 years	Acute kidney injury, dyspnoea, edema and proteinuria	SPE negative, altered sFLC ratio (kappa=477.5 mg/L, lambda=23.3 mg/L, ratio=20.49)	Light Chain Deposition Disease
Male, 54 years	Severe bone pain in the chest, anemia and thrombocytopenia	Small peak in SPE negative (1.17 g/dL), altered sFLC ratio (kappa=3.22 mg/L, lambda 4025 mg/L, ratio=0.0008)	IgD Lambda Multiple Myeloma Stage 3 ISS

**Conclusions:** Freelite allows us an accurate quantification of serum FLCs in the diagnostic study of patients with suspect of monoclonal gammopathies. Due to the high specificity and sensitivity of this assay, it allows us to detect the presence of small amounts of monoclonal proteins that couldn't have been detected by conventional methods.

**A-316**

**Performance Evaluation of Siemens ADVIA Centaur PIGF and sFlt-1 Assays**

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**Background:** Preeclampsia (PE) is a disease found in pregnant women that is characterized by hypertension and proteinuria. PE is caused by an imbalance of the pro- and anti-angiogenic factors PIGF and sFlt-1, respectively, which can adversely impact the health of both mother and fetus. The rapid and accurate diagnosis of PE is essential for best clinical practice and maternal/fetal care. PIGF and sFlt-1, both produced by the placenta, regulate angiogenesis in the developing fetus. The sFlt-1/PIGF ratio becomes elevated when PE is present; therefore, the sFlt-1/PIGF ratio is a valuable tool to aid in diagnosing PE in pregnant women. Siemens Healthcare Diagnostics (Tarrytown, NY, U.S.) has developed ADVIA Centaur® PE assays\* for detection of PIGF and sFlt-1 in serum and plasma to be used in tandem for a clinically relevant ratio of diagnostic value. The objective of this study was to evaluate the performance and demonstrate the clinical utility of the Siemens ADVIA Centaur PIGF and sFlt-1 assays.

**Methods:** The assays were evaluated on the Siemens ADVIA Centaur Immunoassay System for repeatability and within-laboratory precision, method comparison, linearity, limit of detection (LoD), limit of quantitation (LoQ), calibration interval, onboard stability (OBS), hook effect, and endogenous interferences. The clinical agreement with Roche ELECSYS PE assays with >150 clinically relevant samples was also evaluated. In addition, the clinical sensitivity and specificity of the assays using already-established cutoff ratios were evaluated in early gestational ( $\leq$ 34 weeks) and late gestational periods (>34 weeks) from normal and physician-diagnosed preeclampsia patients.

**Results:** The overall analytical performance of the ADVIA Centaur PE assays was excellent. The PIGF and sFlt-1 assays have ranges of 10-10,000 pg/mL and 30-85,000 pg/mL, respectively. From a clinical accuracy perspective, the ADVIA Centaur PE assays demonstrated acceptable clinical sensitivity and specificity within early and late gestational windows. The repeatability was <7% CV, within-laboratory precision was <8% CV and both were linear within each assay's range with no observed hook effect. The LoD and LoQ were <10 pg/mL (PIGF) and <30 pg/mL (sFlt-1). The calibration interval and OBS for both ADVIA Centaur PIGF and sFlt-1 assays were >14 and >28 days, respectively. Less than 10% endogenous interference was observed for all tested interferents for both assays. In addition, the ADVIA Centaur and ELECSYS PE assays showed strong positive (98.2%) and negative (98.8%) agreement by clinical outcome.

**Conclusion:** Overall, the ADVIA Centaur PE assays are a rapid and accurate tool to aid the physician in diagnosing PE. They show very good clinical agreement with

the Roche ELECSYS PE assays. These studies indicate that the ADVIA Centaur PE assays are precise and sensitive for measuring PIGF and sFlt-1 across a wide range of concentrations.

*ADVIA Centaur and all associated marks are trademarks of Siemens Healthcare Diagnostics or its affiliates \*. Under development. Not available for sale. Product availability will vary by country*

### A-317

#### Comparison between two fully automated immunoassay systems for the determinations of IgA anti-tissue transglutaminase antibody in celiac disease

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**Background:** An endoscopy with small intestinal biopsy has generally been considered the gold-standard for celiac disease (CD) diagnosis. With increasing prevalence and awareness of CD, less invasive serologic testing would be of significant value in clinical practice to minimize biopsies especially in pediatric patients. IgA anti-tissue transglutaminase antibody (anti-tTG) is recommended as the initial test for CD. Our laboratory compared and validated the performance between two fully automated immunoassay systems on anti-tTG determinations- the INOVA Quanta Lite h-tTG IgA (INOVA) assay (INOVA Diagnostics, San Diego, CA) and the EliA Celikey IgA (EliA) assay (Thermo-Fischer Scientific, Waltham, MA).

**Methods:** We ran 138 serum samples on both assays and compared the results qualitatively using concordance analysis. The INOVA assays are ran on the ESP600 QuantaLyser automated platform (INOVA Diagnostics, San Diego, CA) and the EliA assays are run on the Phadia 250 analyser (Thermo-Fischer Scientific, Waltham, MA).

**Results:** 19 anti-tTG determinations were negative by both assays and 52 were positive by both for overall agreement of 51.4% when INOVA reference range is set as <20 U/mL. 63 anti-tTG determinations were negative by both assays and 52 were positive by both for overall agreement of 83.3% when INOVA reference range is set as <40 U/mL. 63 anti-tTG determinations were negative by both assays and 69 were positive by both for overall agreement of 95.6% when INOVA reference range is set as <40 U/mL and equivocal range in EliA is interpreted as positive.

**Conclusion:** Both assays demonstrate a better correlation when increasing the cut-off on INOVA. These results imply that performance difference mainly originate from the different cutoffs and implicate for test interpretation and utilization to reduce the number of unnecessary false-positive biopsies.

### A-320

#### Profile analysis of the urea breath test and antibody tests for Helicobacter pylori in a large-scale Korean population

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**Background:** Helicobacter pylori (H. pylori) infection is associated with a wide range of upper gastrointestinal tract disorders including gastric cancer. South Korea has been previously known to have high rates of H. pylori infection with decreasing seroprevalence in the past decade due to improvement of general hygiene and socioeconomic status. The stool antigen test, serological markers, urea breath tests (UBT) are commonly used noninvasive tests whereas gastroendoscopy, rapid urease test and culture are invasive methods for diagnosing H. pylori infection. Among these various testing methods, we analyzed the results of the urea breath test and H. pylori antibodies in relation to the patient population characteristics.

**Methods:** Samples referred to a commercial laboratory for H. pylori testing between January and December 2015 were reviewed. A total of 5,004 samples were tested for <sup>13</sup>C-UBT (HeliFinder<sub>cap</sub>), which utilizes a low-dose <sup>13</sup>C-urea capsule and breath samples collected in test tubes through a straw. Helicobacter antibody testing for IgM was done by ELISA (n=2,882) and IgG was done with a chemiluminescent immunometric assay (n=8,792). The distribution of positive results were compared among age groups and to data previously reported in the literature.

**Results:** The overall positive rate for the UBT method was 21.2% with a slightly lower prevalence in children and teenagers (17.9%, 18 years old or younger) but was not statistically significant (P>0.05). The seropositive rate for all tested samples was higher for IgG (50.9%) than IgM (5.46%), as expected. The seropositivity for IgG increased with age, with the highest positive rate in the 50 to 59 year-old group (61.2%). In contrast, the IgM positive rate was the highest in children and teenagers (11.1%, 18 years old or younger) and showed a steady decrease with increasing age.

**Conclusion:** Due to the relative feasibility of obtaining serum samples in infants and toddlers, testing for H. pylori antibodies was done in all ages (ages 0 to 93) whereas

UBT testing was done in only a handful of children under 10 (n=4). The presence of the H. pylori IgM antibody has been linked to current infection and symptomatic patients and our results demonstrated the presence of an IgM antibody in children as early as 4 years of age. In addition, the overall seropositive rate of IgG in the Korean population was similar to those described in previous studies. The high level of seropositive IgG results may indicate the use of UBT as the noninvasive testing method of choice in areas or countries with a widespread H. pylori infection for discrimination between current and previous infection. The current analysis on a large-scale Korean population demonstrated interesting findings for the H. pylori IgM antibody among age groups, and may suggest the need for follow-up or monitoring in children with positive results.

### A-321

#### Direct measurement of polyclonal IgG4 fab-arm exchange in serum

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**Background:** Human IgG4 molecules are dynamic and have been shown to exchange half molecules to become bi-specific IgG4 hybrid antibodies in a process termed Fab-arm exchange. This process is independent of immunoglobulin light-chain type and can result in the formation of IgG4κλ molecules. Bi-specific molecules cannot cross-link antigen nor elicit lymphoid cell activation. It has been proposed that this mechanism may dampen-down unnecessary inflammatory responses. Measurement of serum IgG4 hybrids has previously been limited to either monoclonal antibodies binding to known antigens or, required the purification of total IgG4 as a first step. Aim: The development of an immunoassay that can directly measure polyclonal IgG4κλ hybrids in serum.

**Methods:** We have developed an enzyme immunoassay (EIA) to detect IgG4κλ hybrids in serum. A specific sheep polyclonal antisera recognizing IgG4λ was raised and processed to specificity. This was used to capture IgG4λ molecules directly from serum. Bound IgG4κλ hybrids were then detected using a total light chain kappa conjugate. Total IgG4 was quantified with a separate EIA. This allowed the calculation of an IgG4κλ hybrid/total IgG4 serum ratio. Purified polyclonal IgG4κλ hybrids were used as a calibrator which was quantified using the Binding Site IgG4 turbidimetric immunoassay. Serum samples from patients with 4 different IgG4-related diseases; Pemphigus/Pemphigoid (n=5), Autoimmune Pancreatitis (n=9) and Primary Sclerosing Cholangitis (n=30, PSC) were analysed and compared to healthy controls (n=95).

**Results:** The concentrations of IgG4κλ hybrids were measured in 95 healthy controls. Using a 1/5,000 sample dilution the assay had a range of 18.5 – 1500 mg/L. The intra assay precision was 8.7, 5.6 and 6.9 %CV using samples that had a high (580 mg/L), medium (85 mg/L) and low (25 mg/L) IgG4 hybrid concentrations, respectively (n=16). A normal range of between 15.2 and 538.8 mg/L of IgG4κλ hybrids was observed in 95 healthy controls. The corresponding total IgG4 concentrations were 17.25 to 1684 mg/L. A first order polynomial fit of total IgG4 against IgG4κλ gave an R<sup>2</sup> of 0.9466 and an intercept of 0.28. The median IgG4κλ hybrid/total IgG4 ratio was 0.33 (range 0.16-0.94). This suggests that the amounts of IgG4κλ hybrid relative to total IgG4 in normal healthy controls appears to be conserved at approximately 30%. This agrees with previous work using serum fractionated IgG4. IgG4κλ hybrid/total IgG4 ratios were lower in IgG4 related disease samples from Pemphigus/Pemphigoid (Mann-Whitney U, p=0.023), and Autoimmune Pancreatitis (p=0.042) compared with healthy controls. However, IgG4κλ hybrid/total IgG4 ratios in PSC samples with either raised or normal IgG4 concentrations were similar to healthy controls.

**Conclusions:** We have created an EIA to directly measure IgG4κλ hybrids and total IgG4 in serum. The ratio of IgG4κλ hybrid/total IgG4 is reasonably conserved in healthy controls but may vary in particular IgG4 related-disease state populations.

### A-322

#### Distribution of antinuclear autoantibody patterns between the year of 2014 and 2015, according to IV brazilian guidelines for autoantibodies on HEp-2 cells

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**Background:** Antinuclear autoantibody (ANA) is performed by indirect immunofluorescence (IIF), and a standard screening method for the presence of ANA in patient serum. With large variety of consensus included the pattern cytoplasmic Rods and Rings, nuclear quasi-homogeneous (QH) and the mixed pattern observed with the presence of anti-CENP-F antibodies. Given this general consensus, we

conducted a survey about the prevalence of all patterns found in a large support laboratory of Brazil.

Methods: Using the database we assessed the patients results for ANA screening between the year of 2014 and 2015. All tests were performed by IIF microscopy method existing patterns, it was necessary to create a Brazilian consensus in order to standardize reading, interpretation, dilution and titration for ANA. Since the first consensus, five main groups of patterns were established - nuclear, nucleolar, cytoplasmic, mitotic apparatus and mixed. In the year 2013, the IV based on the use of HEp-2 cells (Inova NOVA LITE™ IFA Substrate Slide - ANA HeP-2), as substrate and using a screening dilution of 1/160.

Results: We had a total of 956.996 tests, from all regions of Brazil, where 238.433 results were ANA positive and 718.563 negative. The six most frequent ANA pattern obtained were Nuclear fine speckled, Nuclear dense fine speckled, Nuclear coarse speckled, Mixed Pattern, Cytoplasmic reticulated speckled and Nucleolar. All other positive ANA results were distributed in 19 different patterns that are described in table 1.

Conclusion: The most frequent ANA pattern present in 10,022% patients was the Nuclear fine speckled, that can be seen in healthy individuals, but either is associated to patients with immune rheumatic disease. Due to the high sensibility of IIF assay and the great variability of patterns described, a correct clinical interpretation of ANA screening and complementary confirmatory tests are essential for physicians assertivity in diagnostic and treatment of immune rheumatic disease.

Distribution ANA patients accordingly to the pattern on the ANA-HEP2 x Brazilian 4th consensus.					
Pattern	Total	%	Pattern	Total	%
Non Reagent	718563	75,085	Cytoplasmic dense fine speckled	1583	0,165
Nuclear Fine Speckled	95910	10,022	Cytoplasmic Fibrillar	1475	0,154
Nuclear dense fine Speckled	61771	6,455	Cytoplasmic few dots speckled	1372	0,143
Nuclear coarse speckled	15891	1,661	Cytoplasmic fine speckled	1248	0,130
Mixed Pattern (Pattern data not available)	15677	1,638	Nuclear Mitotic apparatus (NuMA 1)	1099	0,1115
Cytoplasmic reticulated Speckled	11929	1,247	Cytoplasmic Linear Fibrillar	894	0,093
Nucleolar	10099	1,055	Polar staining speckled	761	0,08
Nuclear centromeric	6756	0,706	Type 2 nuclear mitotic apparatus (NuMA-2)	626	0,065
Nuclear homogeneous	2212	0,231	Mitotic apparatus (Intercellular Bridge)	601	0,063
Nuclear Coarse speckled reticulated	2184	0,228	Pleomorphic speckled nuclear	582	0,061
Multiple Nuclear dots speckled	1817	0,190	Cytoplasmic Rods and Rings	378	0,039
Nuclear quase-homogeneous speckled	1656	0,173	Mitotic spindle apparatus (centriole)	194	0,02
Nuclear membrane	1609	0,168	Anti-vinculin antibody	109	0,011

**A-323**

**Performance of BhCG, Free T4 and Cortisol assays on Abbott's next-generation immunochemistry analyzer**

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**Objective/Background:** To evaluate the analytical performance of representative immunoassays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for detection of analytes in human plasma/serum on the next-generation immunochemistry analyzer. The sample and microparticle are combined in a reaction vessel. Analyte present in the sample binds to the microparticles. In the second step, conjugate is added to the reaction vessel; the contents are mixed and incubated. After washing, pre-trigger and trigger solutions are added to the reaction mixture; the resulting chemiluminescent reaction is measured as relative light units (RLUs). A relationship exists between the amount of analyte in the sample and the RLUs detected by the optical system.

**Methods:** Key performance testing including precision, linearity, limit of quantitation (LoQ), and method comparison were assessed per CLSI protocols. An assay's measuring interval was defined by the range across which acceptable performance for bias, imprecision and linearity was met.

**Results:** Total imprecision, LoQ, and linearity results along with the defined measuring interval are shown for representative assays in the table below. Results versus the on-market comparator assay demonstrated a slope 0.99 - 1.01 and r = 1.00.

Assay	Total %CV	LoQ	Linearity	Measuring Interval
Cortisol Serum	≤ 3.7	1.0 ug/dL	0.4 -67.5 ug/dL	1.0 – 59.8 ug/dL
Cortisol Urine	≤ 7.9	1.0 ug/dL	0.0 -74.3 ug/dL	1.0 – 59.8 ug/dL
Stat Total BhCG	≤ 5.5	2.30 mIU/mL	1.50 to 16,424 mIU/mL	2.30 to 15,000 mIU/mL
Free T4	≤ 3.1	0.4 ng/dL	0.4 to > 7.85 ng/dL	0.4 to 6.0 ng/dL

**Conclusion:** Representative immunoassays utilizing Chemiluminescent Microparticle Immunoassay (CMIA) technology tested on Abbott's next-generation immunochemistry analyzer demonstrated acceptable precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market comparator assay.

**A-324**

**Study of the clinical sensitivity and specificity of autoantibodies in patients with suspect of Rheumatoid Arthritis in Primary Care**

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**Background:** Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic polyarthritis. In 2009, the new RA criteria released by the American College of Rheumatology (ACR) and the European League Against Rheumatism were revised to include the measurement of anti-CCP antibodies to aid in the classification of RA. The aim of our study is to evaluate the diagnostic value of antibodies in primary care in patients with suspected RA to be remit to a specialist in Rheumatology.

**Material and methods:** Anti-CCP antibodies and rheumatoid factor (RF) were measured in 211 patients with suspected RA. The ACR criteria for RA were fulfilled for 106 patients. We study the diagnostic value (sensitivity, specificity, positive predictive value and negative predictive value) for anti-CCP antibodies, RF and their combinations "anti-CCP and RF" and "anti-CCP or RF".

**Results:** Results are shown in the table.

Antibodies	Sensitivity	Specificity	PPV	NPV
Anti-CCP	66	98	95	82
Anti-CCP and RF	60	98	94	80
Anti-CCF or RF	86	81	74	90
RF	81	81	73	87

**Conclusion:** In primary care, the combination "anti-CCP or RF" exhibits the better diagnostic value with the higher sensitivity than the others combinations.

**A-325**

**Prognostic value of anti-CCP antibodies in patients with Rheumatoid Arthritis under treatment with disease modifying antirheumatic drugs**

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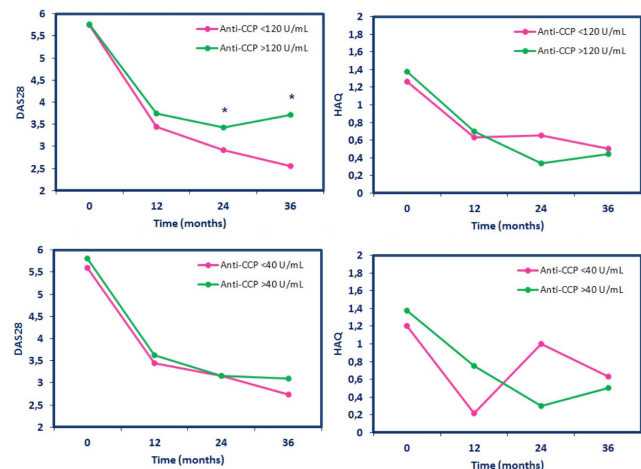
**Background:** Prognostic value of autoantibodies in patients with Rheumatoid Arthritis (RA) is unclear. Studies shows conflicting results regarding the prognostic value of anti-CCP antibodies. The new classification criteria for RA (EULAR/ACR 2010) introduced a weighting for anti-CCP as they are positive at low titers (<3xReference value or VR) and high titers (>3xVR). The aim of our study is to study the prognostic value of anti-CCP antibodies in patients with RA.

**Methods:** 59 patients with recent diagnosed RA and median age of 53 (42-59) years were followed up during 3 years after diagnosis. The patients were treated with disease modifying antirheumatic drugs (DMARDS). Disease activity was assessed by the disease activity score (DAS28) and the functional ability was evaluated by the Health Assessment Questionnaire (HAQ). Patients were stratified in two groups according the cut-offs of 40 U/mL and 120 U/mL (3xreference value). The Wilcoxon test was used to assess changes in parameters (DAS28 and HAQ) during follow up



while to study differences between groups over time we used the Mann-Whitney test was used. A value of  $p < 0.05$  was considered statistically significant.

**Results:** The results for the monitoring of DAS28 and HAQ for the cut-offs of 40 U/mL and 120 U/mL are shown in the figures.



**Conclusion:** A cut-off of 40 U/mL has not prognostic value in the patients with RA according to the evolution of DAS28 and HAQ during the three years of study. However, a cut-off of 120 U/mL allows us to stratify patients in two groups with different response to the treatment according to the DAS28 value.

**A-326**

**Correlation of Beckman Coulter DxI's Total Serum IgE measurement with Siemens Centaur and Thermofisher Phadia platforms**

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**Background:** The measurement of serum IgE aids in the diagnosis and management of atopic allergic disease and hyper-IgE immunodeficiency syndromes. Total IgE is measured on several FDA approved platforms by immunoassay technique. Beckman Coulter DxI is one of the widely used platforms for Total IgE measurement. Recently Beckman Coulter has announced their decision to discontinue the Total IgE assay kit effective 4<sup>th</sup> Quarter, 2016. The objective of this study is to determine whether either Siemens Centaur and/or Thermofisher Phadia instrument platforms are equivalent to Beckman Coulter DxI for measurement of Total serum IgE concentration.

**Method:** Measurement of Total IgE on all three instruments is based on sandwich immunoassay using direct chemiluminescence technology (DxI and Centaur) or fluorescence technology (Phadia). Assay times, Analytical Measuring Ranges (AMR) and Reference Ranges (RR) are 75 min, 0.25 - 3000 IU/mL and 1.3 - 165.3 IU/mL with DxI; 18 min, 1.5 - 3000 IU/mL and <158 IU/mL with Centaur; and 105 min, 2 - 5000 kU/L and <114 kU/L on Phadia, respectively.

Serum samples were measured to determine Total IgE on DxI versus Centaur (N=122) and DxI versus Phadia (N=120). Samples that exceeded AMR on the respective instruments were excluded from the statistical analysis. Results were entered into EP evaluator (version 9.4.0.457; Alternate Method Comparison module) to determine the slope, intercept and correlation coefficient between DxI vs. Centaur and DxI vs. Phadia.

**Results:**

Method Comparison	N	Range (IU/mL)	Slope	Intercept	Corr Coef., R
DxI vs. Centaur	107	1.21 - 2517.34 (AMR)	0.974	1.932	0.9862
	82	1.21 - 188.10 (RR)	1.015	-1.402	0.9950
DxI vs. Phadia	112	2.38 - 3641.28 (AMR)	1.034	-2.553	0.9973
	86	2.38 - 180.83 (RR)	1.055	0.105	0.9866

**Conclusion:** Overall both Centaur and Phadia platforms presented an equivalent performance to Beckman DxI platform for measurement of Total serum IgE.

**A-327**

**Recognition of the dense fine speckled (DFS) pattern remains challenging: Results from an international internet based survey**

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**Background:** The dense fine speckled (DFS) pattern as detected by indirect immunofluorescence (IIF) on HEp-2 cells has been associated with several inflammatory diseases but is most commonly observed in individuals that do not have a systemic autoimmune rheumatic disease and even in apparently healthy individuals. Consequently, the accurate identification and correct reporting of this IIF pattern is of utmost importance which has been recognized by several international study groups for the detection of antinuclear antibodies. The DFS pattern is included in the International Consensus on Antinuclear Antibody (ANA) Pattern (ICAP, <http://www.anapatterns.org/>) referred to pattern AC-02. The objective of this study was to use an internet-based survey to assess how accurately the DFS IIF pattern was recognized by experienced technologists.

**Methods:** High resolution digital IIF images were captured using the automated IIF NOVA View instrument (Inova Diagnostics, San Diego, CA). Ten images were posted in an anonymous, international, internet-based interpretive survey as completed by IIF technologists. Four of the images in the survey were from previously characterized serum samples with classic anti-nuclear antibody (ANA) IIF patterns (nucleolar, centromere, homogeneous, and speckled) and two of the images were from samples with a DFS IIF ANA pattern and monospecific anti-DFS70 antibodies as determined by a chemiluminescence immunoassay. The remaining four images were from sera with the classic IIF ANA patterns referred to above and mixed with the anti-DFS70 positive sample. The survey included multiple choice selections: homogeneous, DFS, centromere, nucleolar, speckled, other, or unrecognizable.

**Results:** 125 of the 230 participants who completed the survey had diverse levels of experience in IIF pattern recognition on HEp-2 cells ranging from <1 year to >10 years experience (average >10 years). Participants had a high concordance in correctly classifying the classical ANA IIF patterns: ranging from 95.2% for centromere to 74.4% for nucleolar patterns. The unmixed DFS pattern was recognized with significantly lower accuracy (~50%;  $p < 0.05$ ). However, less than 10% correctly identified mixed patterns derived from the sera containing clinically relevant and anti-DFS70 antibodies.

**Conclusions:** Recognizing the DFS ANA IIF pattern and mixed IIF patterns composed of DFS + clinically relevant ANA poses a significant challenge. Consequently, it seems imperative that specific immunoassays are needed to confirm the presence of anti-DFS70 antibodies before definitive results are reported to clinicians.

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**A-329**

**A Novel Method in Developing Allergen-Specific IgE Artificial Positive Control Serum**

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**Background:** Serum allergen-specific IgE testing is the most frequently used method in vitro diagnosis of allergy. Positive control sera, important in developing and manufacturing such tests, are very limited availability from natural sources, e.g., patient serum samples. Here we report an innovative method to prepare allergen-specific IgE artificial positive control sera that can be used to prepare calibrators, controls and serum panel for assay development and manufacture to ensure the lot-to-lot consistency.

**Methods:** Specific allergen extracts including E1 (Cat dander), F2 (Milk), W1 (Ragweed), I6 (Cockroach, German), and M6 (*Alternaria alternata*) purchased from various vendors were used to immunize healthy animals and obtain anti-sera. The allergen-specific IgG antibody from animals, purified through the protein A affinity column, was coupled to the human IgE Fc fragment and form the IgG-IgE conjugate. Titers of the IgG-IgE conjugate were evaluated by HOB CLIA-4G Allergy and the results were compared with ImmunoCAP.

**Results:** Five positive artificial control sera have been successfully prepared. The sIgE values of E1, F2, W1, I6 and M6 measured by HOB CLIA-4G Allergy were 914 kU/L, 520 kU/L, 81.4 kU/L, 549 kU/L and 94.9 kU/L, respectively, while the values by ImmunoCAP were 865 kU/L, 565 kU/L, 92.8 kU/L, 470 kU/L and 84.5 kU/L,

respectively. The results showed that these allergen-specific IgE positive control sera have been successfully prepared with high sIgE titers. These artificial IgE sera perform as good as human specific IgE sera in our study.

**Conclusion:** We reported a novel and high-quality method to prepare allergen-specific IgE positive control sera. This method can be easily applied to prepare high-titer and stable positive control sera for various allergens, especially for the alternative replacement of rare allergen-specific IgE control serum. It can be used to manufacture calibrators, controls, and serum panels to keep lot-to-lot consistency well controlled and is easy to scale up. This standardized and reproducible method will greatly contribute to the quality of allergen-specific IgE in vitro diagnostic.

### A-330

#### A Sensitive and Quantitative Method for the Determination of anti-dsDNA IgG antibody on the HOB BioCLIA-1200 Automated Immunoassay Analyzer

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**Background:** Anti-dsDNA is one of the primary auto-antibodies present in patients with systemic lupus erythematosus (SLE). At present, the main methods of detecting anti-dsDNA antibodies are ELISA, indirect immunofluorescence (IIF), radioactive immunoassay (Farr), and dot immuno-gold filtration assay (DIGFA). Farr is considered to be the golden standard for detection of anti-dsDNA antibodies, while it has isotope radioactive. Recently, the innovative HOB anti-dsDNA testing, coupling with the fully automated, random-access BioCLIA 1200 system has been launched.

**Methods:** In this study, the analytical performances of HOB anti-dsDNA Kits including LOD, intra-assay, inter-assay, linearity, accuracy were evaluated. 300 clinical samples from disease group with SLE (n=100) and healthy individuals (n=200) were collected and measured by Farr, HOB anti-dsDNA Kits and ELISA (an internationally renowned manufacturer).

**Results:** 300 clinical samples were analyzed to determine the concordance among Farr, ELISA and HOB anti-dsDNA kits. Compared with the result of Farr, the clinical samples analyzed by ELISA, the data showed positive agreement = 58.0% (58 / 100), negative agreement = 98.5% (197 / 200), while the clinical samples analyzed by HOB anti-dsDNA kits, the data showed positive agreement = 86.0% (86 / 100), negative agreement = 98.0% (196 / 200). The performance results of HOB anti-dsDNA kit were shown in Table 1.

**Conclusions:** The results showed HOB anti-dsDNA kits has good precision, wider dynamic range compared with ELISA. There was poor correlation between Farr and ELISA in detecting anti-dsDNA antibodies in patients with SLE. However, HOB anti-dsDNA kits offered a better clinical relevance and technical performance for SLE diagnosis compared with ELISA.

The analytical performance of HOB anti-dsDNA kits					
Company	LOD (IU/mL)	Intra-Assay (%)	Inter-Assay (%)	Linearity (IU/mL)	Accuracy (%)
HOB	0.03	5.30	6.57	1-800	101.5-103.8
ELISA	1.00	4.40	7.20	10-800	/

### A-332

#### Performance Evaluation of a New, Stable, Ultrasensitive Chemiluminescent Substrate-APSH™

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**Background:** Chemiluminescence immunoassay (CLIA) is a highly sensitive technology compared to Enzyme-linked immunoassay (ELISA), Radioimmunoassay (RIA) and Immunofluorescence Assay (IFA). Substrate plays an important role in the sensitivity of CLIA. There are several commonly used luminescent substances including luminol, isoluminol, acridinium ester, and 1, 2-dioxetane compound. The 1, 2-dioxetane compound can be triggered by alkaline phosphatase (AP) and emit sensitive chemiluminescence. The 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane (AMPPD) has been widely used in different automatic chemiluminescence systems such as those from Siemens, Beckman Coulter, BioMerieux and Olympus. Recently, an innovative & Ultrasensitive HOB AP substrate-APSH™ was developed, and its performance was evaluated by the fully automated, random-access BioCLIA 1200 chemiluminescence immunoassay system. **Methods:** The substrate has been reformulated with: 1) AMP, a buffer to hold

pH basic at 9.8±0.02; 2) AMPPD, a substrate, when subjected to AP cleavage, emits photos as light; 3) sodium fatty alcohol polyoxyethylene ether carboxylate, an anionic surfactant, was coupled with a series of fluorescent compounds (denoted as HOBEP series compounds, HOBEP-1 to HOBEP-5). 4) chemiluminescence enhancer. With the newly formulated substrates, critical assay performance parameters such as LOD, stability and reaction kinetics were assessed by BioCLIA 1200, and compared with an international renowned manufacturer (Company B) substrate. **Results:** The new chemiluminescent substrate-APSH™ has a background signal lower than 200 relative light units (RLU), and its detection sensitivity reaches 10<sup>-19</sup> mol AP. The LOD of HOB TSH kit with APSH™ substrate can lower down to 0.005 ~ 0.012 IU/mL, while that of Company B's is 0.026 IU/mL. APSH™ showed very stable for 18 months at room temperature as its signal retention was above 95% in real-time stability study. The longer term study is still in progress. In addition, APSH™ has a similar reaction kinetics curve to Company B that signal strength reached a plateau at 15 min and duration up to 120 min, while APSH™ has higher signal strength than that of Company B.

**Conclusion:** The new chemiluminescent substrate-APSH™, with high strength and sensitivity, long duration time and long-term stability, satisfies the need of clinical testing on fully automated instruments. It showed an outstanding performance and as good as or better than Company B. The in-house manufacture capability also greatly reduces the cost. In summary, APSH™ is considered as a highly stable and ultrasensitive AP substrate for chemiluminescence immunoassay systems such as HOB BioCLIA-1200.

### A-334

#### Anti-nuclear antibody testing by indirect immunofluorescence and a multiplexed immunoassay: Clinical performance of discordant results against chart review by a Rheumatologist.

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**Background:** Testing for anti-nuclear antibodies (ANA) by indirect immunofluorescence (IFA) using Hep2 cells is labor-intensive and expensive and suffers from poor specificity and high inter- and intra-laboratory variability. As a result, many laboratories have moved away from IFA testing and are relying on automated solid-phase assays for the detection of ANA. However, IFA is touted as the gold standard and many clinicians and laboratories are wary of ANA results from non-IFA assays. In this study, we assess the agreement between ANA IFA and a multiplex solid-phase immunoassay (Bio-Rad BioPlex® 2200) results over a one year period and compare all discordant results from patients seen at a rheumatology clinic to the presence or absence of connective tissue disease (CTD) as determined by a Rheumatologist. **Methods:** Results from patients tested for ANA simultaneously with IFA and BioPlex between May 1, 2011 and April 30, 2012 were analyzed for concordance. Patients with discordant results (ANA+/BioPlex- or ANA-/BioPlex+) had their electronic medical records reviewed by a Rheumatologist to identify the presence of CTD (subdivided into those meeting classification criteria and those with incomplete, but clinically suggestive, presentations) at the time of discordant results until their most recent visit up to mid 2015. **Results:** A total of 1,206 patients had ANA assessed simultaneously by both methodologies. The overall agreement between the two assays was 76.3% (920/1,206). Of the 286 discordant results, 230 were rheumatology patients whose charts were reviewed in detail. 160 were IFA+/BioPlex- and 70 were IFA-/BioPlex+. The negative BioPlex result was clinically concordant in 39/160 cases (24.4%), reflecting an absence of autoimmune disease despite IFA positivity (with titres up to >1:640). The positive BioPlex result was clinically concordant in 61/70 cases (87.1%); 38 (62.3%) of which had established CTD meeting accepted classification criteria. In total, the BioPlex result reflected the patient's clinical presentation in 100 of the 230 charts reviewed (43.5%). **Conclusions:** The IFA and BioPlex assays showed good agreement (76.3%). Among the patients with simultaneously discordant ANA results, 80.4% (230/289) were rheumatology patients subjected to chart review. Of these, the BioPlex agreed with clinician impression 43.5% of the time (100/230). These findings suggest equivalency in the clinical performance of the BioPlex and IFA methods, although neither can be considered a true gold standard.



## A-335

**Cryoglobulinemia typing using Matrix Assisted Laser Desorption/Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS).**

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**Background:** Cryoglobulins are immunoglobulins that precipitate in a reversible- and temperature-dependent process at temperatures below 37°C. There are three main types of cryoglobulinemias. Type I consists of a monoclonal immunoglobulin, typically IgG or IgM and rarely IgA. Type II and III cryoglobulinemias are also known as mixed-type containing more than one immunoglobulin type in the cryoprecipitate. Type II is characterized by a monoclonal and a polyclonal fraction while type III is associated with polyclonal immunoglobulins. In some instances the presence of cryoglobulins does not have clinical consequences. However, in cryoglobulinemias, the insoluble globulins can be associated with weakness, purpura, arthralgias, peripheral gangrene, vasculitis, nephropathy and neuropathy, with symptoms depending on the type of cryoglobulinemia. Consequently, typing is integral to the diagnosis and management of cryoglobulinemias. Cryoglobulinemias are diagnosed by cooling serum to 4°C for up to 7 days in order to observe a protein precipitate which re-dissolves upon warming to 37°C. Cryo-precipitates are then typed using IFE. We recently demonstrated that serum monoclonal and polyclonal immunoglobulin species can be identified from immunoglobulin-enriched samples using MS detection with higher sensitivity. Here, we show that this protocol can be optimized in order to type cryoglobulinemias using MALDI-TOF MS.

**Methods:** 82 residual waste cryoglobulin samples were analyzed using IFE and MALDI-TOF MS, including 35 type I, 30 Type II, and 17 Type III. Serum cryoprecipitates were washed in saline and immunoglobulins from each sample were enriched in separate purifications using nanobody-coupled sepharose beads against IgA, IgG, IgM,  $\kappa$  and  $\lambda$ . Immunoglobulins were eluted with 5% acetic acid and reduced with Tris (2-Carboxyethyl) Phosphine (TCEP) to separate heavy and light chains before MALDI analysis. Saturated  $\alpha$ -cyano-4-hydroxycinnamic acid dissolved in 50% acetonitrile/5water +0.1% trifluoroacetic acid was used as the matrix. MS data were collected using a Microflex LT MALDI-TOF mass spectrometer (Bruker Daltonics) in positive ion mode.

**Results:** 80% of the type I cryoglobulinemia samples analyzed agreed between IFE and MALDI-TOF MS. Similarly, 81% of the type II cryoglobulinemia samples analyzed agreed between IFE and MALDI-TOF MS. Discordant results included; two type III samples observed to have a monoclonal protein by MALDI-TOF MS (IgG- $\lambda$  and IgM- $\lambda$ ), but not by IFE, six samples where IFE and MALDI-TOF MS identified discrepant monoclonal proteins in Type I or Type II cryoglobulinemias, and multiple samples where MS could not assign a immunoglobulin type based on either low signal intensity or non-specific carryover at the purification step.

**Conclusion:** Cryoglobulinemias can be typed using MALDI-TOF MS with up to 80% agreement with IFE. Our initial study suggests feasibility of using the described MS method for cryoglobulinemia typing will require further optimization in order to reduce non-specific carryover at the nanobody purification step. MS detection is attractive based on the potential for automation and reagent cost savings.

## A-337

**Evaluation of autoantibody screening and disease-specific methods for autoimmune disease diagnosis**

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**Objective:** To characterize differences from three facilities utilizing different methods for measuring autoantibodies.

**Relevance:** Investigations of autoimmune disease include measurement of autoantibodies with immunofluorescence microscopy and specific solid phase immunoassays. A better understanding of the performance of different autoantibody methods is needed to employ an effective autoimmune disease diagnostic strategy tailored to the local patient population and scope of service.

**Methodology:** 100 patient serum samples were analyzed for autoantibodies by computer-aided indirect immunofluorescence (IFA) by two methods (Euroimmun and Inova) at different sites and by multiplex bead-based assays (BioPlex and TheraDiag/FIDIS) at three different sites. Agreement between methods were assessed qualitatively (positive/negative) and by computer-aided interpretation of patterns as shown by immunofluorescence microscopy. Specific autoantibody results were

interpreted both quantitatively (between BioPlex instruments) and qualitatively (between BioPlex and TheraDiag/FIDIS).

**Results:** Comparison of the BioPlex instruments at two different locations employing 44 of the 100 patient samples demonstrated good concordance (95.5%) and quantitative correlation (slopes ranging 0.82 - 0.98 with R<sup>2</sup> values between 0.840 - 0.998 for specific autoantigens). Conversely, BioPlex and IFA (Euroimmun) methods showed relatively poor concordance at 61.0 %; however IFA methods showed good agreement for staining pattern (80.8 %). Common autoantigens between BioPlex and TheraDiag/FIDIS demonstrated good, although varied, concordance for the various analytes (81 - 97 %). The differences between BioPlex and TheraDiag/FIDIS were attributed to differences in autoantigen preparations.

**Conclusions:** This study demonstrated some differences between platforms for autoantibody methods. BioPlex instruments were consistent between two sites; however comparison of IFA and specific autoantibody methods from two different manufacturers demonstrated differences. This is attributed to the general lack of standardization of autoantibody analysis diagnostic kits as it pertains to autoantigen preparations. Given these differences, it is important that laboratory results should be interpreted in the context of the clinical picture and that specific diagnostic performance of autoantibody methods should be taken into account for effective delivery of laboratory services.

## A-338

**Islet Autoantibody Selection for Population Screening**

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**Background:** When type 1 diabetes (T1DM) becomes preventable, screening for islet autoantibodies will be used to detect individuals at risk for T1DM. Such individuals would then undergo further testing prior to receiving preventative therapy. Presently there are 5 major autoantibodies that are used to define risk for T1DM: ICA, IAA, GADA, IA-2A and ZnT8A. The goal of this study was to determine if any one autoantibody or set of autoantibodies are more common in sera positive for at least one autoantibody that might allow more focused testing (e.g., single or dual screening versus screening for all 5 autoantibodies). **Methods/Results:** The initial analysis involved 71 sera tested for all 5 autoantibodies. These samples were submitted for the presumed evaluation of islet autoimmunity. Of these 71 sera, 33.8% sera were positive for at least one autoantibody. Positivity rates were: ICA 22%; IAA 8.4%; GADA 23%; IA-2A 22%; and ZnT8A 29%. IAA positivity was not significantly less common than that of the other autoantibodies (P = 0.1). Only 16.7% of sera were positive for a single islet autoantibody and 83.3% of sera were positive for =>2 autoantibodies. For samples with at least one positive autoantibody, the most common pattern was positivity for ICA, GADA, IA-2A and ZnT8A in 33% of samples. Because of this limited sample size, 854 sera were analyzed that were all tested for ICA, IAA, GADA and IA-2A. 72.8% of sera were positive for at least one autoantibody. Positivity rates were: ICA 37%; IAA 45%; GADA 52%; and IA-2A 50%. Compared to the other 3 autoantibodies, ICA was less common (P <0.0001). GADA, compared to the other 3 autoantibodies, was more common (P <0.0001). GADA and IA-2A were equally common (P = 0.38). If the goal of testing were to identify 2 islet autoantibodies in a subject's serum, by screening for GADA and IA-2A and not further testing people with both GADA and IA-2A or neither GADA or IA-2A, only 1.1% of double autoantibody positive subjects could be missed with a 36% reduction in test volume versus testing for all 4 islet autoantibodies in all subjects. **Conclusion:** We conclude that while GADA is positive more often than other autoantibodies, the differences in positivity rates are minimal and it is not possible with the available data to choose a single "best" panel of tests to screen for islet autoimmunity. Future analysis of cascade testing may allow more prudent use of testing to recognize islet autoimmunity.

## A-339

**Using anti-GD1a, GD1b, GB1b antibodies to diagnose Guillain-Barre syndrome, a case report.**

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Guillain-Barre syndrome (GBS) is a disease presenting as rapid-onset muscle weakness. Although the exact etiology remains unknown, it is believed that GBS is an autoimmune disease caused by antibodies against the peripheral nerves. Diagnosis of GBS is dependent on clinical evaluation and electrophysiological studies. It is debated if, for challenging cases, ancillary laboratory tests on serum anti-ganglioside antibodies

may help establishing the diagnosis. The patient is a 78-year-old man with past medical history of hypertension and prostate carcinoma status post brachytherapy. He presented to the Emergency Department with progressive difficulty with ambulation for one week. Physical examination showed weakness in all four extremities. Guillain-Barre syndrome was suspected. Head CT-scan and MRI scan were negative for acute pathology. Electromyography showed no evidence of myopathy, but scattered evidence of neurogenic pathology. Tests on serum anti-Jo, anti-acetylcholine receptor, anti-MUSK and anti-campylobacter antibodies were negative. Patient was given intravenous immunoglobulin treatment for five days and improvement in muscle strength was observed. In order to support the clinical diagnosis of Guillain-Barre syndrome, serum tests on anti-GD1a, GD1b, and GB1b was performed by ELISA and the results turned out to be negative. Gangliosides are components of cell membranes found mostly in nervous system where their function may include regulation of neuronal growth and development. Anti-ganglioside complexes (GSCs) antibodies, including anti-GD1a/GD1b, GD1a/GM1, GD1b/GT1b, GQ1b/GM1, and GQ1b/GD1a antibodies are associated with several syndromes of peripheral neuropathy including but not limited to GBS. The sensitivity of this antibody test for GBS reported in the literature is low. One study showed that only about 38% of GBS patients have detectable titer of serum anti-ganglioside antibodies, while anti-GM1 antibody is seen in about 20-30% of the GBS patients. Another study showed that only 17% of 234 GBS patients had serum anti-GSCs antibodies detected. High titer of anti-GM1 antibody (> 1:2000) may be seen in GBS, as well as multifocal motor neuropathy, motor neuron disease, and non-specific idiopathic neuropathy, therefore, the antibody test may not be specific for GBS too. Our current patient did not show the presence of anti-GD1a, GD1b, and GB1b antibodies in the serum, even though he had clinical and electrophysiological suspicion for GBS, which is in accordance with the low sensitivity of the test reported in the literature. Therefore, the increased cost of this serological test may not justify its use due limited sensitivity and specificity.

#### A-340

##### **Pleiotropic effects of 4-Hydroxynonenal on Oxidative Burst and Phagocytosis in Neutrophils**

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Metabolic control of cellular function is significant in the context of inflammation-induced metabolic dysregulation in immune cells. Generation of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide are one of the critical events that modulate the immune response in neutrophils. When activated, neutrophil NADPH oxidases consume large quantities of oxygen to rapidly generate superoxide and hydrogen peroxide, a process that is referred to as the oxidative burst. These ROS are required for the execution of efficient processing and removal of phagocytized cellular debris and pathogens. In chronic inflammatory diseases, neutrophils are exposed to increased levels of oxidants and pro-inflammatory cytokines that can further prime respiratory burst responses. However, the effects of metabolism on the oxidative burst in response to ROS inducing conditions are not well understood. The purpose of this study was to investigate the effects of the nonenzymatic lipid peroxidation product 4-hydroxynonenal (HNE), a diffusible reactive aldehyde generated endogenously under conditions of increased oxidative stress, on neutrophil oxidative burst and cellular proteins. The oxidative burst was determined in freshly isolated healthy donor neutrophils using 13-phorbol myristate acetate (PMA) and the extracellular flux analyzer. Neutrophils pretreated with HNE caused a significant decrease in oxidative burst response in a dose dependent manner. Mass spectrometric analysis of alkyne-HNE treated neutrophils followed by click chemistry detected modification of a number of cytoskeletal, metabolic, redox and signaling proteins that are critical for NADPH oxidase mediated oxidative burst. These modifications were confirmed using a candidate immunoblot approach for critical proteins of the active NADPH oxidase enzyme complex (p47phox subunit, Rac1 of the NADPH oxidase etc.) and glyceraldehyde phosphate dehydrogenase, a critical enzyme in the metabolic regulation of oxidative burst. Taken together, these data suggest that lipid peroxidation-induces damage to NADPH oxidase and other critical cellular proteins and enzymes. These mechanisms may contribute to the immune dysregulation associated with chronic pathological conditions.