Tuesday, August 1, 2017

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

Immunology

A-332

Paroxysmal, but not persistent, atrial fibrillation is associated with increased levels of transforming growth factor beta1

A. E. Stanciu[1], R. G. Vatasescu[2], M. M. Stanciu[3], M. Dorobantu[2]. *[1]Institute of Oncology, Bucharest, Romania, [2]Clinic Emergency Hospital, Bucharest, Romania, [3]University Politehnica of Bucharest, Bucharest, Romania*

Background: Inflammation plays an important role in the genesis and maintenance of atrial fibrillation (AF). The precise mechanism and signaling pathways involved in structural remodeling and atrial fibrosis are still unknown. We aimed to assess the extent of inflammation in pathogenesis of different types of AF.

Methods: Twenty-five patients with paroxysmal AF, 30 patients with persistent AF and 20 healthy control subjects were enrolled in the study. Peripheral blood samples were collected before catheter ablation of pulmonary veins. Serum levels of NT-proBNP, IL-6, TGF-beta1, MMP-9 and TIMP-1 were measured by ELISA.

Results: NT-proBNP, IL-6, TGF-beta1 and MMP-9/TIMP-1 ratio were higher in AF patients than in controls (P<0.001). NT-proBNP and IL-6 levels were higher in persistent AF than in paroxysmal AF (172.5±67.6 pmol/L vs. 122.2±56.7 pmol/L, P=0.02 and 14.7±9.8 pg/mL vs. 8.5±4.2 pg/mL, P=0.003). TGF-beta1 and MMP-9/TIMP-1 ratio were lower in persistent AF than in paroxysmal AF (16.7±1.8 ng/mL vs. 25.2±3.2 ng/mL, P=0.006 and 3.1±1.2 vs. 5.2±3.4, P=0.002). TGF-beta1 inversely correlated with NT-proBNP (r= - 0.84, P<0.001). Higher levels of IL-6 and NT-proBNP in persistent AF than in paroxysmal AF, suggest that IL-6 and NT-proBNP may be related to the burden of AF. Atrial fibrogenesis is accompanied by a biphasic response, an early increase of TGF-beta1 and MMP-9/TIMP-1 ratio in paroxysmal AF and a later loss of TGF-beta1 and MMP-9/TIMP-1 ratio in persistent AF.

Conclusion: The later loss of TGF-beta1 may result from the reduction of pulsatile stretch of atrial cardiomyocytes, due to the spreading of cardiac fibrosis or from antifibrotic functions of NT-proBNP. Our data suggest that fibrosis progresses, despite compensatory changes in the TGF-beta1 signaling pathway.

A-334

Discrepancies between two immunoassays for the determination of MPO and PR3 autoantibodies

Q. Sun[1], B. Calderon[2], Z. Zhao[1]. *[1]NIH, Bethesda, MD, [2]Eli Lilly and Company, Indianapolis, IN*

Background: Testing for autoantibodies to myeloperoxidase (MPO) and proteinase 3 (PR3) is part of an anti-neutrophil cytoplasmic antibodies (ANCA) panel test that aids the diagnosis of small-vessel vasculitis, inflammatory bowel disease, as well as systemic autoimmune diseases such as Lupus. Several immunoassays have been approved by the FDA for the measurement of MPO-ANCA and PR3-ANCA in patient serum. Here we characterized the differences between two automated immunoassays at three facilities for measuring MPO and PR3 autoantibodies.

Methods: 117 patient serum samples were analyzed for MPO and PR3 autoantibodies. The INOVA Quanta Lite IgG assay (INOVA Diagnostics) were performed on the DSX workstation (DYNEX Technologies) at site 1 and site 2 and the BioPlex 2200 Vasculitis Panel were performed on the BioPlex 2200 testing platform (Bio-Rad Laboratories) at site 3. The results were compared both qualitatively (between two methods) and quantitatively (between INOVA assays).

Results: Comparison of the INOVA assay at two different facilities employing 36 patient samples demonstrated high concordance (97.2% for MPO and 94.4% for PR3) and quantitative correlation (Deming regression R^2=0.973 for MPO and R^2=0.935 for PR3). Conversely, INOVA and BioPlex methods showed relatively poor concordance at 70.4% for MPO (n=81; 95%CI: 59.7% to 79.2%) and at 76.5% for PR3 (n=81; 95%CI: 66.2% to 84.4%). The comparison results were shown in Table 1.

Conclusion: This study demonstrated low concordance between two methods for MPO-ANCA and PR3-ANCA measurements. INOVA Quanta Lite IgG assays were consistent between two sites; however, comparison of INOVA and BioPlex multiplex system demonstrated differences. This is consistent with the general lack of standardization of antigen-specific immunoassays and different mechanisms employed by these two techniques. Given the discrepancies, the performance of different autoantibody immunoassays should be taken into consideration when interpret the MPO-ANCA and PR3-ANCA results.

Table 1. Concordance of different methods of MPO and PR3 autoantibodies

Concordance (95% CI)	INOVA (site 1)	
	MPO	PR3
INOVA (site 2)	97.2% (85.8 to 99.5%)	94.4% (81.9 to 98.5%)
BioPlex (site 3)	70.4% (59.7 to 79.2%)	76.5% (66.2 to 84.4%)

A-335

Investigating the regulatory role of a negative checkpoint molecule, VISTA in endothelial cells.

M. Salehi, I. Radichev, A. Savinov. *Children's department, Sanford Research, Sioux Falls, SD*

Background: Peripheral tolerance is crucial to maintain tolerance against self-antigens and fine tune immune responses. The mechanism of immune tolerance and homeostasis is mediated by a family of co-stimulatory and co-inhibitory molecules that regulate T cell function. Targeting these molecules and their pathways have been promising for therapeutic approaches in autoimmune diseases and cancer. V-set domain Ig Suppressor of T Cell Activation (VISTA) is a recently identified co-inhibitory member of this family, which suppresses T cell immune responses. Based on preclinical studies, VISTA deficiency augments acute inflammation and predisposes individuals to autoimmune disorders. In our preliminary investigation, we identified a sub-population of endothelial cells (ECs) with high level of VISTA expression residing exclusively in secondary lymphoid organs. Considering the previous studies that signifies the important role of specific subsets of ECs from lymph nodes in the induction of immune tolerance, we pursued to further characterize the population of VISTA-expressing ECs.

Methods: We performed immunofluorescent staining on frozen tissue sections of lymph nodes from mice to characterize VISTA-expressing EC. To separate ECs into subpopulations based on VISTA expression, Fluorescence-activated cell sorting (FACS) was applied. RNA from sorted cells were further analyzed using Real-Time Quantitative Reverse Transcription PCR for expression of peripheral tissue antigens (PTAs). To study the function of VISTA expression by ECs, isolated mouse T cells were labeled with CellTrace Violet fluorescent dye and cocultured with VISTA-expressing ECs. Subsequent proliferation of T cells was analyzed by Flow Cytometry.

Results: We found that VISTA-expressing cells belong to both lymphatic and blood subsets of ECs which control immune cells migration to the lymph nodes. In NOD*scid* mice with impaired T and B cell development, VISTA expression was lost on the blood EC subset but was maintained on lymphatic ECs. We also found that VISTA expression is induced postnatally through adulthood contrary to PD-L1, a similar co-inhibitory molecule. Furthermore, FACS-sorted ECs from lymph nodes of NOD (model for Type I Diabetes) and DBA (control) mice were evaluated for the expression levels of various PTAs that are associated with immune tolerance. In RNA of NOD mice, PTAs were up-regulated in VISTA^{high} ECs which associates with their inflammatory phenotype. Finally, coculture of VISTA-expressing ECs with T cells resulted in suppression of T cell proliferation.

Conclusion: Our results suggest that the lymph node microenvironment could have a role in regulation of VISTA expression by ECs. Moreover, our results show that VISTA-expressing ECs in the lymph nodes are favorably situated to tolerize immune cells particularly T cells during their migration into and out of the lymph nodes. VISTA-expressing ECs could function as a checkpoint regulator for T cell function and therefore play a role in controlling autoimmune disorders.

A-336

Serum Anti-PLA2R Antibody and Glomerular PLA2R Deposition in Chinese Patients with Membranous Nephropathy: A Retrospective Study

L. Pang, H. Li, J. Du, N. Duan, Y. Liu, D. Yu. *Peking University First Hospital, Beijing, China*

Background: M-type phospholipase A2 receptor (PLA2R) is the major target antigen in primary membranous nephropathy (MN). Previous studies have evaluated the diagnostic value of serum anti-PLA2R antibody. However, the correlation of serum

anti-PLA2R antibody and glomerular PLA2R deposition, and their association with clinical characteristics need to be further evaluated.

Methods: In total, 960 inpatients who performed serum anti-PLA2R antibody measurement between August 2015 and December 2016 were initially reviewed retrospectively. The patients who did not performed renal biopsy were excluded. Thus, 284 patients with renal biopsy proven MN and 427 patients with biopsy proven non-MN were included. Of all the MN patients, 83 patients were clinically ruled out for secondary MN. Therefore, 136 patients were selected as inception group because serum anti-PLA2R antibody and glomerular PLA2R antigen were simultaneously detected. We examined serum anti-PLA2R antibody by ELISA and glomerular PLA2R deposition by immunohistochemical staining in inception group.

Results: Positive serum anti-PLA2R antibody and glomerular PLA2R deposition were seen in 58.8% (80/136) and 95.6% (130/136) patients respectively ($p < 0.001$). None of the patients with other glomerular diseases were positive for serum anti-PLA2R antibody. In our study, the specificity of serum anti-PLA2R antibody for PMN is 100% and the sensitivity is 58.8%. Although the Spearman's correlation coefficient is 0.18 ($P = 0.109$), the high level of serum PLA2R antibody was related to the strong expression of glomerular PLA2R antigen. Proteinuria, serum total protein, serum albumin, serum creatinine and eGFR had significant differences between patients with serum anti-PLA2R antibody and those without. There were no significant differences in any clinical biomarkers between glomerular PLA2R deposition-positive and -negative patients. Serum anti-PLA2R antibody levels were correlated with serum albumin, serum creatinine, eGFR and proteinuria. Glomerular PLA2R deposition intensities were weakly correlated with proteinuria. Unexpectedly, there was a positive correlation rather than a negative correlation between glomerular PLA2R deposition intensity and eGFR.

Conclusion: Serum anti-PLA2R antibody is more closely correlated with disease activity and renal function than glomerular PLA2R deposition.

A-338

Variability in Approach to Initial Antinuclear Antibodies (ANA) Testing and Follow-Up Testing: a Survey of Participants in the College of American Pathologist's Proficiency Testing Program

S. J. Naides[1], J. R. Genzen[2], G. Abel[3], M. Shan[4], C. Bashleben[4], M. Q. Ansari[5]. *[1]Quest Diagnostics Nichols Institute, San Juan Capistrano, CA, [2]ARUP Laboratories Inc, Salt Lake City, UT, [3]Lahey Hospital & Medical Center Burlington, Burlington, MA, [4]College of American Pathologists, Northfield, IL, [5]Cleveland Clinic, Cleveland, OH*

Background: A 2010 American College of Rheumatology (ACR) position paper stated that the indirect immunofluorescence assay (IFA) on HEp-2 cell substrate is the "gold standard" method for ANA testing and that laboratories performing other methods should state the method used and describe its performance parameters. Our aim was to identify current practices in initial non-IFA testing and reflex testing directed by an initial positive IFA screen.

Methods: Supplemental questions were sent to laboratories participating in the College of American Pathologist's proficiency testing program for ANA as part of the Special Immunology S-A Survey 2016 to determine the practice of ANA testing. Of 5847 kits distributed, 1206 (21%) responded to the questionnaire; 942 were in the United States and 264 were international.

Results: Of 669 laboratories performing an initial ANA IFA, only 33% offer reflex testing. Of those offering a reflex option, the follow-up testing was by specific autoantibody(ies) (multiple responses allowed) by enzyme linked immunosorbent assay (ELISA) in 47%, by IFA in 25%, multibead immunoassay (mbead) in 25%, and by ELISA (but not to a specific analyte) in 6%. In 14%, follow-up testing was reported as "other." Of 669 laboratories reporting, 21% initially screened by ELISA. When an ELISA was used as initial testing, 39% of 216 responding laboratories reported using HEp-2 cell lysate as substrate, 4% a lysate of the HEp-2000 cell line (which is engineered to over express SSA antigen), 25% a mixture of specific antigens and 32% "other" substrate. When an ELISA was used for initial ANA screening, results were reported as positive/negative in 67%, in units by 21%, optical density by 3%, and "other" by 9%; 69% of 178 laboratories reported reflexing a positive ELISA to IFA, 24% to specific autoantibody ELISAs, 7% to specific autoantibody IFA, 4% to specific mbead, and 8.4% to "other." When mbeads were used in 134 reporting laboratories, specificities of antibodies tested were 100% SSB, 99% SSA, 99% Jo-1 and RNP, 98% Scl-70, 96% double stranded DNA, 87% centromere B, 58% ribosomal P, 55 Sm/RNP, 44% histone, 8% centromere A, 2% single stranded DNA,1% centromere F and 7% "other." An internal fluorescence standard in mbead testing was included by only 74% of laboratories, and a positive control in each assay by 52% and daily by 44% of laboratories. When initial ANA testing was performed by mbead, a positive screen result reflexed to specific analytes in only 57% of 133 reporting laboratories.

Conclusion: Marked variation exists in reflex strategies following a positive initial ANA IFA. In those labs performing an initial screen by methods other than IFA, choice of method and specificities of autoantibodies tested vary widely, which can affect results reported. With the variability in approach to initial ANA testing and follow-up testing, clinical practice would benefit from the development of uniform laboratory guidelines promulgated collaboratively by the laboratory and rheumatology communities.

A-339

Effects of Glycated Albumin on Inducing Injury and Inflammation in Human Proximal Tubular Epithelial Cells

N. Duan, H. Li, Y. Liu, L. Pang. *Peking University First Hospital, Beijing, China*

Background Glycated albumin (GA) serves as a clinically useful index for monitoring glycemic status and it is also associated with pathogenesis of renal complications in diabetes mellitus. This study aim to investigate the effects of GA on the expression of kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL) and inflammatory cytokines in human proximal tubular epithelial cells (HK-2 cells).

Methods The HK-2 cells were treated with the different concentration of GA (0.5 mg/ml; 1.0 mg/ml) and albumin (Alb) (0.5 mg/ml, 1.0 mg/ml; 5 mg/ml, 10 mg/ml) for 12h, 24h and 48h. The mRNA expressions of KIM-1 and NGAL were detected by real-time PCR. The releases of KIM-1 and NGAL in the supernatants were detected by ELISA. The concentrations of vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF-α), interleukin-8 (IL-8) and soluble intercellular cell adhesion molecule-1 (sICAM-1) were detected by cytometric beads array method.

Results Compared with control and Alb groups, the mRNA levels of KIM-1 and NGAL in HK-2 cells were significantly up-regulated at 12 h, 24 h and 48 h after GA treatment ($P < 0.05$); the protein levels of KIM-1 and NGAL released in supernatants of GA-treated cells were significantly higher than those in control group and Alb groups at the same time points ($P < 0.01$); GA groups also had significantly higher levels of sICAM-1, VEGF and IL-8 than control group at each time point ($P < 0.05$).

Conclusions GA can up-regulate the expression and release of KIM-1 and NGAL and promote the secretion of inflammatory cytokines which could cause damage to renal tubules, suggesting that GA may reflect the diabetic renal involvement.

A-340

Abbott Alinity i Sigma Metrics and Precision Profiles for Immunoassays

V. Petrides[1], S. Westgard[2], S. Schneider[1], J. Herzogenrath[3], A. Orzechowski[1]. *[1]Abbott, Abbott Park, IL, [2]Westgard QC Inc, Madison, WI, [3]Abbott, Wiesbaden, Germany*

Introduction: Assay performance is dependent on the accuracy and precision of a given method. These attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for 13 immunoassays tested on the Alinity i-series. Additionally, precision profile charts were created for a subset of assays to compare the precision performance of the assays tested using the Alinity i-series and the ARCHITECT *i* system.

Methods: A sigma value was estimated for each assay and was plotted on a method decision chart. The sigma value was calculated using the equation: $sigma = (\%TEa - |\%bias|) / \%CV$. A precision study was conducted at Abbott on each assay using the Alinity i-series per CLSI EP05-A2 where assay controls and panels were tested in replicates of 2-3 during 2 runs each day for 20 days, and the data were used to calculate a within-laboratory %CV. To estimate the %bias, 40-100 serum samples with concentrations spanning the assay's measuring interval were tested in duplicate at Abbott on the Alinity i-series and ARCHITECT $i2000_{SR}$ systems. The mean concentration of the Alinity i-series results were regressed versus the mean ARCHITECT $i2000_{SR}$ results and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated at a medical decision point. For a subset of assays, a precision profile chart was created by plotting the within-laboratory %CV values versus the mean concentration values for both the Alinity i-series and the ARCHITECT *i* system, where the ARCHITECT *i* system

within-laboratory %CV and mean concentration values were obtained from the assay package inserts.

Results: The method decision chart showed that a majority of the assays demonstrated at least 5 sigma performance at or near the medical decision point. The precision profile charts of the within-laboratory %CV results for the Alinity i-series overlaid with the ARCHITECT *i* system showed similar performance across the subset of assays evaluated.

Conclusion: Sigma metrics, method decision charts, and precision profile charts can be valuable tools for evaluating and comparing product performance by providing a comprehensive understanding of expected assay performance. The majority of Alinity i-series immunoassays had sigma values greater than 5. The precision performance on the Alinity i-series and ARCHITECT *i* systems was comparable for the subset of assays for which a precision profile was created. Laboratorians can use these tools as aids in choosing high-quality products, further contributing to the delivery of excellent quality healthcare for patients.

A-341

Breaking Free From the Ratio: Analytical Performance of an Immunoenrichment-Coupled MALDI-TOF MS Detection Method for Monoclonal Immunoglobulin Free Light Chains

L. Sepiashvili, M. C. Kohlhagen, D. L. Murray. *Mayo Clinic, Rochester, MN*

Background: Bence Jones proteins or monoclonal immunoglobulin free light chains (FLCs) hold an important supportive role in diagnosis, prognosis, and monitoring of multiple myeloma. An immunonephelometric serum FLC assay that quantitates kappa (K) and lambda (L) light chains unbound to heavy chains is among the most sensitive assays for monoclonal immunoglobulin (M-protein) detection. However, the presence of an M-protein, represented by an abnormal K/L FLC ratio (K/L<0.26 or >1.65), often cannot be corroborated by other clinical lab methods, including serum protein electrophoresis (SPEP) and immunofixation electrophoresis (IFE). Non-secretory multiple myeloma, light chain multiple myeloma, primary systemic amyloidosis, and light chain deposition disease often are only detected by the K/L FLC ratio (rFLC) due to relatively low abundance M-proteins in circulation. Moreover, the rFLC can be abnormal in the absence of an M-protein or vice versa.

Objective: To overcome the above limitations, the objective of this study was to develop and evaluate the analytical performance of an immunoaffinity enrichment-coupled MALDI-TOF mass spectrometry (MS) method for direct detection of monoclonal FLCs.

Methods: Residual sera (n=129) were evaluated in 3 cohorts varying based on the presence of an M-protein by IFE and rFLC: IFE-negative and normal rFLC (Cohort 1, n=36), IFE-positive and abnormal rFLC (Cohort 2, n=38) , and IFE-negative and abnormal rFLC (Cohort 3, n=55). For analytical sensitivity assessment, residual sera containing K or L M-proteins (>0.3 g/dL, n=4) were serially diluted into a polyclonal serum pool until undetectable. All sera were analyzed by conventional lab methods for M-protein detection, including: (i) SPEP (Helena laboratories), (ii) IFE (Sebia), and (iii) immunonephelometry for FLCs (Binding Site reagent, Siemens BN II analyzer). Serum FLC immunoenrichment was performed with sepharose beads conjugated with polyclonal antibodies that have high specificity towards FLCs and low cross-reactivity with light chains bound to heavy chains. Total IgG, IgM, IgA, K light chain, and L light chain nanobody immunoenrichments were performed as previously described (Mills JR *et al*, 2016, Clinical Chemistry). Immunoenriched specimens were reduced to dissociate heavy and light chains and subjected to MALDI-TOF MS (Bruker Microflex™) in automated acquisition mode. Mass spectra were interrogated for M-proteins and isotypes.

Results: Cohort 1 and 2 MALDI-TOF MS results were 100% concordant with IFE and rFLC results. In Cohort 3, 36 of 55 (65%) sera had evidence of monoclonal immunoglobulins or monoclonal FLCs by MALDI-TOF MS, supporting abnormal rFLC results obtained by immunonephelometry. These results suggest that MALDI-TOF MS can help resolve the majority of cases with dichotomous IFE and rFLC results. However, the remaining 19 sera did not contain detectable M-proteins by MALDI-TOF MS despite abnormal rFLC, corroborating negative IFE. FLC immunoenrichment coupled to MALDI-TOF MS was on average 4.3 (range=2-5) and 34 (range=16-80) times more sensitive than rFLC and IFE towards monoclonal light chain detection, respectively. Notably, FLC M-proteins remained detectable by MALDI-TOF MS when rFLCs became normal following serial dilution.

Conclusions: Immunoenrichment using anti-FLC antibodies followed by MALDI-TOF MS is a highly sensitive and specific approach for detection of monoclonal abnormalities frequently undetectable by current routine laboratory methods.

A-342

Diagnosis of red meat allergy with antigen-specific IgE tests in serum

J. R. Brestoff, M. A. Zaydman, M. G. Scott, A. M. Gronowski. *Washington University, Saint Louis, MO*

Background: Red meat allergy is a rare tick-associated hypersensitivity reaction to galactose-α-1,3-galactose (α-gal) and is characterized by anaphylaxis, angioedema, urticaria and/or gastrointestinal symptoms occurring 3-6 hours after ingesting red meat such as beef, pork, or lamb. Due to the poor sensitivity of skin prick tests with commercial meat extracts (20-40%), the primary diagnostic tools available for diagnosis of red meat allergy are quantification of α-gal-, beef-, pork-, and/or lamb-specific IgE in serum or plasma. However the sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) for these tests have not been previously reported.

Methods: To address this, we performed a systematic literature search and identified 22 articles that reported individual patient data for these tests from 135 patients with red meat allergy and 37 controls. The gold standard for diagnosis was expert consensus by the articles' original authors. None of these articles used antigen-specific IgE test results to define disease or control status. These individual patient data were extracted and aggregated in a dataset representing patients from the United States (25.0%), Austria (16.9%), Sweden (12.8%), other European countries (25.6%), Australia (16.9%) and China (0.6%).

Results: We found that measurement of α-gal-specific IgE using the bovine thyroglobulin (bTG) ImmunoCAP method had the best overall sensitivty (100%) and specificity (92.3%) for diagnosis of red meat allergy. Measuring biotinylated α-gal-specific IgE (α-gal biotin) using the streptavidin (SA)-CAP method or beef- or pork-specific IgE using ImmunoCAP were also effective tests with high sensitivities (89-92%) and variable specificities (65-82%). Receiver Operating Characteristic (ROC) analyses showed that the areas under the curve (AUC) for α-gal bTG (AUC 0.97), α-gal biotin (AUC 0.93) and beef (AUC 0.86) IgE tests were significantly higher than those of pork (AUC 0.72), lamb (AUC 0.68) and total IgE (AUC 0.42) (all comparisons P<0.05). Since the prevalence of red meat allergy is not known and likely varies widely geographically, Bayesian statistics were used to calculate PPVs and NPVs for all possible pre-test probabilities for these tests. This analysis demonstrated that the α-gal bTG test had the highest PPV and NPV for any given pre-test probability, whereas lamb IgE and total IgE had essentially no diagnostic value for red meat allergy.

Conclusions: These findings indicate that the α-gal IgE test using the bTG ImmunoCAP method is the most useful for establishing a diagnosis of red meat allergy, although the biotinylated α-gal IgE test using the SA-CAP method and beef IgE by ImmunoCAP are also effective tests.

A-343

Alinity i Assay Performance of Representative Immunoassays

C. Rudolph, K. Krishnan. *Abbott Diagnostics Division, Abbott Park, IL*

Objective: To evaluate the analytical performance of representative immunoassays that utilize Chemiluminescent Microparticle Immunoassay (CMIA) technology for the quantitative determination of analytes in human plasma/serum on the Alinity i, Abbott's next-generation immunoassay analyzer. The Alinity i analyzer is a high throughput instrument testing up to 200 tests per hour. The sample and paramagnetic microparticles are combined in a reaction vessel. Analyte present in the sample binds to the antibody-coated microparticles. The mixture is washed. Antibody acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of antigen in the sample and the RLUs detected by the optical system.

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

Results: Total imprecision, LOQ, linearity, and defined measuring intervals are shown for the representative immunoassays in the table below. Results versus an on-market comparator assay are also shown.

Assay	Total %CV	LOQ	Linearity	Measuring Interval
CA19-9XR	≤ 10	2.06 U/mL	1.66 to 1623.36 U/mL	2.00 to 1200.00 U/mL
CA125 II	≤ 4	0.6 U/mL	1.1 to 1138.4 U/mL	1.0 to 1000.0 U/mL
CA15-3	≤ 6	0.6 U/mL	0.3 to 925.4 U/mL	0.5 to 800.0 U/mL
HE4	≤ 4	2.0 pmol/L	3.2 to 1741.3 pmol/L	20.0 to 1500.0 pmol/L
BNP	≤ 5	5.0 pg/mL	2.1 to 5271.1 pg/mL	10.0 to 5000.0 pg/mL
Estradiol	≤7	26 pg/mL	12 to 1230 pg/m/L	26 to 1000 pg/mL

Conclusion: Representative immunoassays utilizing Chemiluminescent Microparticle Immunoassay (CMIA) technology tested on the Alinity i immunoassay analyzer demonstrated acceptable precision, sensitivity and linearity. Method comparison data showed excellent agreement with on-market immunoassays.

A-344

Performance evaluation of Nanopia KL-6 assay in interstitial lung diseases

E. Cho, K. Park, H. Hong, E. Shin, W. Lee, S. Chun, J. Song, H. Koo, H. Koo, K. Do, W. Min. *Asan medical center, SEOUL, Korea, Republic of*

Background: Krebs von den Lungen-6 (KL-6), which is a mucin-like glycoproteins excreted from type II alveolar pneumocytes when these cells are injured. KL-6 has been reported to serve as a sensitive marker for monitoring disease activity and predicting the prognosis of interstitial lung diseases (ILD). The aim of the present study was to evaluate the analytical and clinical performance of Nanopia KL-6 assay (Sekisui Medical Co. Ltd. Tokyo, Japan) based on latex-enhanced immunoturbidimetry method. **Methods:** From March to October 2016, 260 patients diagnosed with ILD were enrolled in this study. All patients with ILD underwent HRCT and pulmonary function test (PFT). We used 113 samples and 200 samples for disease and healthy control, respectively. The evaluation consisted of determination of the precision, linearity, method comparison with ELISA kit (EIDIA, Tokyo, Japan), sensitivity and specificity and correlation with HRCT findings or PFTs. The HRCT findings were graded on a one to six scale based on the classification system. **Results:** The total CV for low and high level quality control materials were below 2% at each concentration. Acceptable linearity was observed in their respective reportable ranges. Correlation analysis of KL-6 indicated that results of the Nanopia KL-6 assay were comparable to ELISA [correlation coefficients (r) = 0.979]. Using a ROC curve, the optimal cutoff point of KL-6 was 350 U/mL with a sensitivity and specificity of 73.9% and 98.0%, respectively, and the area under the curve was 0.953. Serum KL-6 levels was positively correlated with the extent of involvement, traction bronchiolectasis and ground-glass attenuation on the HRCT. In the comparison of all ILD patients' subgroups, significantly higher levels of KL-6 were determined in the idiopathic pulmonary fibrosis (IPF) or connective tissue diseases-related ILDs (CTD-ILD) than other groups. KL-6 levels were negatively correlated with PFTs [FVC, DL_{CO}, TLC and 6MWT]. In IPF patients, there were statistically significant correlations with all PFT results, but in nonspecfic interstitial pneumonia and hypersensitivity pneumonitis groups, some of the test measurements showed a good correlation with KL-6. **Conclusion:** The overall analytical and clinical performance of Nanopia KL-6 assay is acceptable for the monitoring of disease progression in clinical practice. Therefore, KL-6 serve as useful non-invasive biomarker to assess the disease severity in patients with ILD.

A-347

Complete IFA Automation vs Manual IFA reading for Anti-Nuclear Antibody testing. An Evaluation of the AESKU HELIOS Instrument and software

T. Alexander, L. Define, P. Agner. *Summa Health, Akron, OH*

Background: In 2009, the American College of Rheumatology (ACR) issued a position statement for anti nuclear antibody (ANA) testing, stating, in part, "The ACR supports the immunofluorescence antinuclear antibody (ANA) test using Human Epithelial type 2 (HEp-2) substrate, as the gold standard for ANA testing". However, the immunofluorescent assay is labor intensive and requires trained, experienced individuals to properly interpret the fluorescent staining patterns. Thus there is a need for IFA automation, both for the preparation of the slides and for IFA pattern reading and interpretation. These needs may be addressed by the AESKU HELIOS instrument and software. The HELIOS is an automated system for immunofluorescence processing with image capturing. This instrument performs both the slide preparation and, with an integrated fluorescence microscope and software, can interpret HEp-2 staining patterns. This study evaluated the performance of the HELIOS Automated IFA System with AESKUSLIDES ANA HEp-2-Gamma from AESKU Diganostics and compared the HELIOS to AESKUSLIDES ANA HEp-2 slides processed and read manually. Specifically we:

1. Compared HELIOS results to manual results at 1:40 and 1:80 serum dilutions,
2. Compared the HELIOS pattern recognition interpretation to the interpretations of two trained laboratorians
3. Determined clinical sensitivity and specificity of the HELIOS based on the manual method.

Methods: We analyzed 556 clinical serum specimens from the clinical categories listed below at 1:40 and 1:80 dilutions using the AESKUSLIDES HEp-2 gamma kit. Slides were read by two independent,readers blinded to the identity of specimens in each well and one HELIOS.

- Normal Controls: 80
- Rheumatic Diseases associated with ANA positivity: 264
- Other diseases not associated with ANA positivity: 201
- Diverse samples (selected due to ANA positivity or rare patterns): 11

Results: Method comparison with manual assay % Total Agreement (95% CI) between HELIOS and manual method at 1:40 dilution

- Positive/negative total agreement = 96.0% (94.6-97)
- Pattern agreement = 94.2% (92.2-95.7)

at 1:80 dilution • Positive/negative total agreement = 93.6% (92.2-94.7)

- Pattern agreement = 93.6% (91.3-95.3)

Clinical studies: To determine clinical sensitivity and specificity, a cohort of 460 clinically characterized samples were tested.

- 264 Rheumatic Diseases associated with ANA positivity (CTD + AIL)
- 196 Other diseases not associated with ANA positivity
- 30 normal control samples

HELIOS vs. manual method at 1:40

- SLE: sensitivity = 88.9% vs. 87.8%
- CTD+AIL: sensitivity = 86.0% vs. 86.4%
- Specificity: 67% vs. 65.5%

HELIOS vs. manual method at 1:80

- SLE: sensitivity = 78.9% vs. 82.2%
- CTD+AIL: sensitivity = 78.8% vs. 86.4%
- Specificity: 77% vs. 78%

Conclusion: The HELIOS demonstrated excellent agreement with manual slide reading for both positive/negative interpretations and pattern recognition. The HELIOS also performed similarly to the manual method in terms of clinical sensitivity and specificity.

A-348

(K+L) Index: a new biomarker of disease activity in Rheumatoid Arthritis

J. Garcia de Veas Silva[1], M. López Vélez[1], A. Espuch Oliver[1], L. Pérez Albaladejo[1], N. Barbosa de Carvalho[2], J. García Lario[1], T. De Haro Muñoz[1]. *[1]Complejo Hospitalario Universitario de Granada, Granada, Spain, [2]The Binding Site, Barcelona, Spain*

Background: Rheumatoid Arthritis (RA) is a systemic inflammatory autoimmune disease characterized by chronic polyarthritis and bone destruction. B cells are implicated in the inflammatory events of the RA by producing antibodies and a policlonal excess of serum free light chains kappa (K) and lambda (L). Thus, the sum of the serum levels of K and L could be associated with B cells activation and the inflammatory activity of the RA. The aim of the study is to evaluate the "(K+L) index" as new biomarker of activity disease in patients with RA based on the following studies: i) to compare the (K+L) index between healthy donors and RA patients, ii) to evaluate the diagnostic accuracy of (K+L) index in active disease and iii) to correlate (K+L) index with the disease activity index DAS28 and others biomarkers of RA (ACPA, PCR, FR and VSG).

Methods: study based on 69 healthy donors (12 male:57 female with a median age of 50 (43-57) years old) and 73 patients with RA (13 male:60 female with a median age of 58 (50-70) years old; 28 patients in remission and 45 patients with active disease). Disease activity was evaluated using DAS28 score. Pairwise comparison was carried out with Mann-Whitney U test, Receiver Operator Curve (ROC) was used to evaluate

the efficacy of (K+L) index and Spearman correlation analysis was used for assessing the relationship between quantitative variables. A p value <0.05 was considered statistically significant. Statistical analysis was made with Prism 6.0.

Results:

Objective 1: Serum levels of (K+L) index in patients with RA were significantly higher than healthy controls: 37.86 (30.90-45.79) mg/L vs. 24.99 (19.14-29.20) mg/L, respectively (p<0.0001). Between RA patients; serum levels of (K+L) index in patients with active RA were significantly higher than those of patients in remission: 38.29 (34.23-53.05) mg/L vs. 33.73 (30.11-39.10) mg/L, respectively (p=0.018).

Objective 2: The area under curve in patients with active RA was 0.855 (95% CI 0.786-0.908) for (K+L) index. The optimal cut-off determined by ROC curve for (K+L) index was 32.98 mg/L. The maximum sensitivity and specificity were 80% and 85%; respectively.

Objective 3: A good linear correlation was found between (K+L) index and DAS28 score (r=0.503; p<0.0001) and VSG (r=0.270; p=0.02). No significantly correlation was found with PCR (r=0.09), FR (r=-0.03) and ACPA (r=-0.007).

Conclusions: The levels of serum "(K+L) index" in patients with active RA were significantly higher than those of patients in remission and of healthy controls. An optimal cut-off of 32.98 mg/L allows us to diagnose activity states of the disease. The (K+L) index could be used as biomarker of disease activity in RA.

A-349

Complement activation in systemic lupus erythematosus with antiphospholipid antibodies

R. V. Alexander, T. O'Malley, J. Conklin, C. Ibarra, T. Dervieux. *Exagen Diagnostics, Inc, Vista, CA*

Background. Complement activation is a hallmark of systemic lupus erythematosus (SLE) and leads to formation of cell-bound complement activation products (CBCAPs). We established that C4d split fragment bound to erythrocytes (EC4d) and B-cells (BC4d) is a sensitive and specific biomarker for SLE. Because subjects with SLE often develop auto-antibodies to phospholipid complexes (APL), we evaluated the relationship between complement activation in APL positive and negative subjects with SLE or other diseases.

Methods. Blood was collected in EDTA from subjects with SLE (n = 541, 91% females, mean age 41 years), other diseases (n = 615, 85% females, mean age 55 years, inclusive of 287 with rheumatoid arthritis) and normal healthy volunteers (NHV, n = 210, 65% females, mean age 41 years). EC4d and BC4d expression was determined by flow cytometry and reported as net mean fluorescent intensity (MFI). Positive CBCAPs consisted of positive EC4d (> 14 net MFI) and/or BC4d (> 60 net MFI). Anti-cardiolipin IgG, anti-beta-2-glycoprotein 1 IgG, or anti-phosphatidylserine/prothrombin complex IgG antibodies were determined using ELISA (INOVA Diagnostics, San Diego, CA). Presence of APL antibodies consisted of any of these 3 antibodies above manufacturer cutoff. Groups were compared by Fisher's exact test.

Results. CBCAPs yielded 61% sensitivity and 89% specificity in distinguishing SLE from other diseases (99% specific in NHV). APL positivity was higher in SLE than in other diseases (40% vs. 17%). APL positive SLE subjects had higher incidence of CBCAPs positivity than APL negative (77% vs. 51%, p<0.0001) (Table). Similar trends were observed in the group of other diseases and NHV.

Conclusions. APL occur at higher rate in SLE than other diseases. APL antibodies may contribute to complement activation and CBCAPs formation in SLE.

	APL positivity (%)	CBCAPs positivity in APL positive (%)	CBCAPs positivity in APL negative (%)
SLE, n = 541	40%	77%	51%
Other diseases, n = 615	17%	18%	10%
NHV, n = 210	14%	7%	0%

A-350

Evaluation of a novel Latex agglutination immunoturbidimetry assay kit, H. pylori-Latex "SEIKEN", for detection of anti-*H.pylori* antibody in serum and plasma.

A. Ishii. *Denka Seiken Co.,Ltd., Chuo-Ku, Tokyo, Japan*

Background:*Helicobacter pylori* (*H. pylori*) is a helix-shaped Gram-negative microaerophilic bacterium existing in human gastric mucosa. It is well-known that *H. pylori* infection causes chronic gastritis and significantly increases risks of a gastric ulcer, a duodenal ulcer, and gastric cancer. *H.pylori* infection can be diagnosed by various methods such as endoscopic atrophy, rapid urease test (RUT), microscopy, culture, urease breath test (UBT), urine or serum/plasma antibody tests, and a stool antigen test. Among the choices, serum/plasma antibody test is widely used in Japan since it is comparatively less invasive and less expensive than the others and also allows analysis of a large number of samples with an auto-analyzer. We have developed H. pylori-Latex "SEIKEN" (Denka Kit), a novel latex agglutination immunoturbidimetry assay kit, for detecting anti-*H. pylori* antibody in human serum and plasma. The analysis by Denka kit is a simple procedure, which comprises measurement on an auto-analyzer capable of accommodating two-reagent assay, and is completed within 10 minutes. In this study, the basic performances and accuracy to clinical diagnosis of the Denka kit were evaluated.

Methods: The Denka kit was evaluated for the following basic performance: (1) within-run precision, (2) lower detection limit, (3) linearity, (4) prozone, (5) interferences, (6) comparison between serum and plasma collected from the same patients, and (7) accuracies to ELISA kits in market (IBL, Bio-Rad, Monobind). In addition, the results of 159 patients sera tested by the Denka kit were evaluated in comparison to clinical diagnosis, which was determined from the overall judgment of the endoscopic atrophy, the RUT, and the culture.

Results: (1) Samples with two different levels (5.1 U/mL and 21.1 U/mL) were measured in 20 replicates. The coefficients of variation (CVs) were within 1.0-2.0%. (2) The lower detection limit was 1.0 U/mL. (3) Linearity was observed up to 100 U / mL. (4) No prozone phenomenon was observed up to 400U/mL. (5) No interference effects were observed for hemoglobin up to 500 mg / dL, bilirubin up to 30 mg / dL, and chyle up to 2000 FTU. (6) Consistency was shown by 98.2% (55/56) between serum and EDTA plasma, and by 100.0% (39/39) between serum and heparin plasma. (7) The Denka kit had 98.8%, 93.3%, and 92.2% accuracies to the three ELISA kits. The sensitivity, specificity, and accuracy of the Denka kit compared with the clinical diagnoses were 93.1% (81/87), 97.2% (70/72), and 95.0% (151/159), respectively.

Conclusion: The Denka kit showed excellent basic performances and high diagnostic accuracies confirmed with clinical diagnoses. Compared to other diagnostic methods, the Denka kit allows rapid measurements of a large number of specimens in a fully automated manner. The results of this study suggest that not only the Denka kit is highly efficient for diagnosis of *H. pylori* infection but also useful for mass screening in the preliminary stage such as endoscopic examination.

A-351

Adjusted serum free light chain reference ranges on the SPAPlus platform

T. Theparee, R. C. Benirschke, H. Lee. *NorthShore University Healthsystem, Evanston, IL*

Background: Serum free light chain measurements by nephelometry have been useful in the monitoring of disease progression or treatment success in patients with multiple myeloma. The original free light chain reference ranges measured on the Beckman IMMage 800 platform were the manufacturer's ranges. Our hospital recently switched to the Binding Site SPAPlus platform which did not have separate reference ranges as per the manufacturer. During validation with patient comparison studies, the SPAPlus platform demonstrated higher values when compared to the IMMage platform, establishing the need for new in-house reference ranges.

Objective: To establish and validate new kappa, lambda light chains, and K/L ratio reference ranges for our patient population on the Binding Site SPAPlus platform.

Methods: Patient sera were tested on the Binding Site SPAPlus Freelite Human Lambda and Kappa Free Kit according to the manufacturer's instructions. Forty-seven patient sera were tested on a Beckman Coulter IMMage 800 Immunochemistry platform concurrently with the Binding Site SPAPlus platform. Deming Regression for concentrations less than 5 mg/dL was used to determine theoretical reference ranges on the SPAPlus platform. An additional 82 normal patient sera with normal serum protein electrophoresis (SPE) results were used to validate these reference ranges. These 82 normal patients were without plasma cell neoplasms, hematologic malignancies, autoimmune diseases, active infections and had normal creatinine, calcium, liver enzymes, IgG, IgA, and IgM concentrations.

Statistical analysis was performed using R statistical software.

Results: The following table shows the old, new, and validated reference ranges.

Conclusion: Kappa, lambda light chains and K/L ratio reference ranges that were previously used on the Beckman IMMage 800 platform could not be translated to the Binding Site SPAPlus platform. We propose new reference ranges for the Binding Site SPAPlus platform based on our study.

Old, new, and validated reference ranges			
	Old Reference Ranges	**New Reference Ranges**	**Validated Reference Ranges**
Kappa Light Chains (mg/dL)	0.33-1.94	0.33-3.26	0.37-3.08
Lambda Light Chains (mg/dL)	0.57-2.63	0.46-2.71	0.51-1.99
K/L Ratio	0.26-1.65	0.49-2.54	0.60-2.26

A-352

Evaluation of the Stability of Centrifuged EDTA Specimens for HIV Serology Testing

N. Tran, A. Hardesty, P. Akl. *University of Oklahoma Health Sciences Center, Oklahoma city, OK*

Background: Human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS) is a major cause of illness and death in the United States. The diagnosis of HIV infection itself carries major personal, social and financial implications. It is therefore critical for both the patient and the physician to be certain of the diagnosis. In our laboratory, routine HIV serology testing is performed in a batched fashion, Monday through Friday. Whole blood specimens are centrifuged as soon as they are received in the laboratory and plasma is separated from the cells within 24hrs. This separation step however, increases the risk of specimen mislabeling, and thus may misclassify patients as either HIV reactive or non-reactive. To eliminate this separation step and thus prevent the risk of specimen mislabeling, we performed a stability study by testing HIV serology on centrifuged primary specimens, without plasma separation, at <24hrs and 4-5days post collection.

Methods: Forty-three patients were included in the study. EDTA whole blood specimens, recommended specimen type for HIV serology in our laboratory, were centrifuged, stored at 2-8°C, and tested at <24hrs and 4-5days. HIV serology testing was performed on both the Advia Centaur-XP (Siemens Healthcare Diagnostics Inc., Tarrytown, NY) and Architect Plus-i2000 (Abbott Diagnostics, Abbott Park, IL).

Results: As shown in Table-1, 46.5% (20/43) of tested specimens were reactive for HIV, while 53.5% (23/43) were non-reactive. The concordance between the <24hrs and 4-5days stored specimens were 100% on both the Advia Centaur-XP and the Architect Plus-i2000. No discordant events were observed.

Conclusion: We conclude that HIV serology testing can be performed up to 4-5days on centrifuged primary EDTA specimens with no change in the results. Therefore, specimens received on the weekend could be tested on the following weekday without the need to separate plasma from the cells, decreasing the risk of specimen mislabeling.

Table-1: HIV test results		Centaur		Architect	
		4-5 days		4-5 days	
		Reactive	Non-reactive	Reactive	Non-reactive
<24 hours	Reactive	20	0	20	0
	Non-reactive	0	23	0	23

A-353

High cut-off haemodialysis and serum free light chains: improving the treatment of patients with Multipl Myeloma and acute kidney injury

P. Menendez Valladares[1], J. M. Maesa[1], J. L. Garcia De Veas Silva[2], N. Barbosa de Carvalho[3], C. Bermudo Guitarte[1]. *[1]Virgen Macarena University Hospital, Seville, Spain, [2]Granada University Hospital Complex, Granada, Spain, [3]The Binding Site Group, Barcelona, Spain*

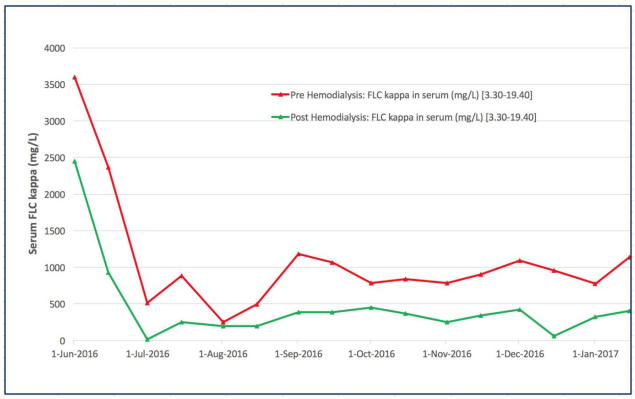
Background: Acute kidney injury (AKI) is present in 15-30% of patients with Multiple Myeloma (MM) and the survival of these patients is highly dependent on the recovery of the renal function. The effective elimination of serum free light chains (sFLC) with the application of haemodialysis with high cut-off membranes (HCO-HD) alongside with chemotherapy is associated with an improvement in the renal function.

Methods: A 60 year-old woman diagnosed of IgG Kappa MM ISS-II was admitted to the hospital due to relapse of the disease. During his stay, an AKI was detected with creatinine=5.35 mg/dL, urea=70 mg/dL, an EFGR=8.1 mL/min/1.73 m^2 and an altered sFLC ratio=373.8 (kappa sFLC=3595.95 mg/L). The patient underwent sixteen sessions of HCO-HD to remove sFLC in addition to Bortezomib and Dexamethasone (B/D) treatment. sFLC were measured by turbidimetry using the assay Freelite (The Binding Site, UK). Blood samples were collected pre- and post-HD to determine creatinine and sFLC.

Results: During therapy kappa sFLC levels decreased significantly (see graphic). After sixteen cycles of HCO-HD, kappa sFLC clearance was 89% from 3595.95 mg/L to 404.6 mg/L. This treatment produced an improvement in the patient´s renal function with a decrease of 51.78% in the creatinine serum levels, with an EFGR=19.5mL/min/1.73 m^2. After HCO-HD, the patient continued on conventional haemodialysis and finished the treatment with B/D achieving a partial response (PR) with negative immunofixation; kappa sFLC=404.6 mg/L, sFLC ratio=25.05. Bence Jones proteinuria of 0.2 g/24h and presence of 1.2% of plasmatic cells in bone marrow.

Conclusion: A combination of the efficient and direct removal of the nefrotoxic excess of sFLC using HCO-HD with effective chemotherapy with B/D allowed an efficient reduction of the sFLC levels. sFLC determination by Freelite allowed an accurate and rapid evaluation of the rate of sFLCs decrease, proving useful to monitor the efficiency of the therapy adopted.

adopted.



A-354

Quantitation of Serum Immunoglobulins by Capillary Electrophoresis

C. Wunsch. *University of Miami School of Medicine, Miami, FL*

OBJECTIVE: To enhance the utility of serum protein electrophoresis by developing a method for quantitating IgA, IgG and IgM immunoglobulins from the electrophoretogram (ELP). METHODS: Digital data from 700 clinical serum ELPs were downloaded from a CapillaryS™ analyzer. Values for IgA, IgG, IgM, total κ Ig, and total λ Ig measured on the same ELP specimen were obtained from a BNII analyzer. The adjusted sum of total κ + total λ Ig was required to be within 5% of the sum of G, A, M-Igs for further analysis. Specimens with ELP clonal characteristics were excluded. The majority of remaining 510 ELPs had one or more abnormalities. Seven features - curve heights or areas - of the ELP were chosen for regression studies. Each curve feature was regressed against IgA, IgG, and IgM. Regression yields an equation relating the feature to each Ig. β-Igs were calculated with graphical algorithms. For most ELPs, the β-γ boundary was defined as the valley between the zones. Simplex analysis was chosen to provide an approximate solution to the equations. Simplex analysis requires 4 equations to estimate the IgA, IgG and IgM of an ELP. Python programs and R statistical analysis were used. RESULTS: A simplex needs initialization with an estimate of the Igs for convergence. IgG and IgM were usually approximated from the γ peak height, and IgA from the β-Ig. The smallest R-square for any of the regressions used was 0.88. β-zone Ig, γ-zone Ig, γ-peak height, and γ-zone mid-point height all had R-square values of 0.96 or more. By itself, the γ-peak height is directly proportional to IgG with a correlation of 0.95, but the regressed peak correlation significantly improved to 0.98 due to inclusion of IgM - the IgA coefficient was not significant. Most sets of simplex equations included the β-γ boundary height. Three sets of simplex equations produced correlations better than 0.97 for the IgG and IgA of ELPs, but none produced an IgM correlation better than 0.50. DISCUSSION: The sum of β-Ig and λ-Ig should equal the sum of G, A, M-Igs, thus regression coefficients for β-Ig and λ-Ig should be complements of each other. Their coefficients, determined independently, however, are not exact complements of each other. The placement of the β-γ boundary plays a critical role in the regression of β- and γ-Ig and the height of the β-γ boundary. Large elevations of γ-Ig shift the valley between the β-γ zones upward and toward the anode. This small shift has a large effect on the least squares calculations of regression. The values used to initialize the

simplex are also important. Seeding the best simplex with values within 5 to 10% of the target values produced G, A, M-Ig correlations above 0.95, but the IgM correlation dropped to 0.33 when simple approximations were used. CONCLUSION: Good estimates of IgG and IgA can be extracted from ELPs. With improvements to the above approach, one should also be able to obtain a good estimation of IgM.

A-355

Performance Evaluation of the ADVIA Centaur Lipopolysaccharide Binding Protein (LBP) Assay

V. Taylor[1], N. Smith[1], L. Buchan[1], J. King[1], P. Kelly[1], D. Hovanec-Burns[2]. *[1]Axis-Shield Diagnostics, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics, Tarrytown, NY*

Background: Lipopolysaccharide binding protein (LBP) is a soluble acute-phase protein (65 kDa) that binds to bacterial lipopolysaccharide (LPS) to elicit immune responses and may be used in diagnosis and prognosis of diseases that are induced by exposure to endotoxin, such as sepsis and infectious complications of surgery and trauma. The ADVIA Centaur® LBP assay* for the quantitative measurement of LBP in human serum and plasma is under development by Axis-Shield Diagnostics Inc. for Siemens Healthcare Diagnostics Inc. The objective of this study was to evaluate assay performance for precision, linearity, limit of quantitation (LoQ), interference and cross-reactivity, and method comparison to the IMMULITE® 2000 LBP assay† (Siemens Healthcare Diagnostics Inc.).

Method: The ADVIA Centaur® LBP assay is a sandwich immunoassay that employs direct chemiluminescent technology. LBP is bound to mouse monoclonal anti-LBP antibody-coated particles and is then detected by an acridinium ester-labelled anti-LBP mouse monoclonal antibody. Following incubation, a wash step, and magnetic separation, acidic and basic reagents are added to the reaction mixture, and the resulting chemiluminescence is measured. The method comparison study was performed per CLSI EP-09-A3 using 121 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A, respectively. In addition, following CLSI-EPO7-A2, performance of the assay was assessed against a list of potential interfering substances including hemoglobin, conjugated and unconjugated bilirubin, triglyceride, biotin, cholesterol, IgG, total protein, rheumatoid factor, lipemia, and HAAA. Furthermore, also per CLSI-EPO7-A2, the assay was tested with potential cross-reactants such as human serum amyloid A, IL-6, IL-8, TNFα, and CRP.

Results: Observed range of the assay was up to 120 µg/mL without dilution and up to 240 µg/mL with manual 1:2 dilution. The assay's linearity was observed up to 120 µg/mL. The limit of quantitation was observed at 1.5 µg/mL, with a total error of 19%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 3.3–4.1%. Passing-Bablok procedure comparison of the assay to the IMMULITE 2000 LBP assay gave a slope and intercept of 1.20 and 2.76 µg/mL, respectively. The Pearson's correlation r value was 0.95. The assay demonstrated no significant interference or cross-reactivity from the tested analytes.

Conclusions: The feasibility of an automated LBP assay on the Siemens ADVIA Centaur XP Immunoassay System has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of LBP.

*Under development. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

†Not available for sale in the U.S.

A-356

Transcription Factor YY1 Induces Interleukin-8 Production in Rheumatoid Arthritis via PI3K/Akt Signaling Pathway

J. Lin, Y. He, Q. Ou. *Department of Laboratory Medicine, The First Affiliated Hospital of Fujian Medical University, Chin, Fuzhou, China*

Background: Previous studies have revealed a critical role of YY1 in cancer development and progression. However, whether YY1 has any role in rheumatoid arthritis (RA) remains unknown. This study aims to explore the potential role of YY1 in RA pathogenesis.

Methods: Expression of YY1 was detected by real-time PCR and western blotting. The signaling pathway was done by ingenuity pathway analysis (IPA). Vascular cell adhesion molecule 1 (VCAM-1) and interleukin-8 (IL-8) expression was detected by real-time PCR and ELISA. CIA mice were treated with YY1 related lentivirus to observe the role of YY1 *in vivo*. **Results:** We found that YY1 was over-expressed in RA patients and CIA mice. Blocking of YY1 action with YY1 shRNA lentivirus ameliorated disease progression in CIA mice. We further analyzed the signaling pathway involved by ingenuity pathway analysis (IPA), results showed IL-8 signaling and PI3K/Akt signaling pathway was significantly inhibited by LV-YY1-shRNA treatment. Moreover, we observed that blocking of YY1 reduced IL-8 and VCAM-1 production and neutrophil adhesion via PI3K/Akt signaling pathway. **Conclusion:** In conclusion, YY1 plays a critical role in promoting IL-8 production in RA which contribute to the inflammation of RA via stimulation of neutrophil adhesion. Thus, YY1 is likely a key molecule involved in the inflammation process of RA. Targeting of YY1 may be a novel therapeutic strategy for RA.

A-357

Performance Evaluation of the ADVIA® Centaur Erythropoietin (EPO) Assay*

V. Taylor[1], H. Laird[1], M. Chalmers[1], H. Lang[1], E. Ma[1], J. King[1], P. Kelly[1], J. Lei[2]. *[1]Axis-Shield Diagnostics Ltd, Dundee, United Kingdom, [2]Siemens Healthcare Diagnostics Inc., Tarrytown, NY*

Background: Erythropoietin (EPO) is a glycoprotein hormone that controls erythropoiesis, or red blood cell production. It is a cytokine for erythrocyte (red blood cell) precursors in the bone marrow. Measurement of EPO is used as an aid in the diagnosis of anemias and polycythemias. The ADVIA Centaur® EPO assay* (under development by Axis-Shield Diagnostics Inc. for Siemens Healthcare Diagnostics Inc.) is intended for the quantitative measurement of EPO in human serum and plasma using the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate assay performance for precision, linearity, limit of quantitation (LoQ), and method comparison to the ACCESS EPO assay (Beckman Coulter).

Method: The ADVIA Centaur EPO assay is a sandwich immunoassay that employs direct chemiluminescent technology. EPO is bound to mouse monoclonal anti-EPO antibody-coated particles and is then detected by an acridinium (NSP-DMAE)-labelled anti-EPO mouse monoclonal antibody. Following incubation, a wash step, and magnetic separation, acidic and basic reagents are added to the reaction mixture and the resulting chemiluminescence is measured. In a preliminary study, performance of the ADVIA Centaur EPO assay was evaluated for precision, linearity, limit of quantitation (LoQ), and method comparison to Access EPO (Beckman Coulter). The method comparison study was performed per CLSI EP-09-A3 using 140 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A respectively.

Results: The reportable range of the prototype assay is up to 750 mIU/mL without dilution or up to 7500 mIU/mL with automated or manual 1:10 dilution. Linearity was demonstrated up to 750 mIU/mL. The limit of quantitation was 0.93 mIU/mL, with a total error of 29.1%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 1.55-5.64%. Method comparison of the ADVIA Centaur EPO assay to the ACCESS EPO assay using Passing-Bablok regression gave a slope and intercept of 1.00 and 0.23 mIU/mL, respectively. The Pearson's correlation r value was 1.00. Mean EPO values for the ADVIA Centaur and ACCESS assays were 41.02 and 41.45 mIU/mL, respectively, and sample doses ranged from 0.82 to 751.29 mIU/mL for the ADVIA Centaur EPO assay.

Conclusions: The feasibility of an automated EPO assay on the Siemens ADVIA Centaur XP Immunoassay Systems has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of EPO in human serum and plasma.

*Under development. The performance characteristics of this device have not been established. Not available for sale, and its future availability cannot be guaranteed.

A-358

Characterization and Engineering of Allergenic Materials Using Capillary Immunoblot and Application on BioCLIA® 4G Allergy Assay

S. W. P. Chan[1], L. Trondle[1], S. V. Wetering[1], L. Qian[2], C. Lee[2]. *[1]CRM Biotech, Irvine, CA, [2]HOB Biotech Group, Suzhou, China*

Background: *In vitro* allergen-specific IgE assays, integrated with component-resolved diagnostics (CRD), offer allergists an allergen-sIgE sensitization profile with insight into clinical relevance such asrisk of anaphylaxis versus benign local symptoms. CRD has been developed in various testing platforms, e.g.,BioCLIA® 4G Allergy (HOB Biotech Group, China). CRD uses allergenic molecules for reflex testing in response to a positive result from a traditional extract-based test. Extracts

from different sources may contain varying allergenic component concentrations leading to discrepant results, and allergenic components are often used tosupplement raw allergen extracts to increase test sensitivity. An accurate quantitative method is required to determine and then adjust the component composition to ensure commutable results and lot-to-lot consistency. This study aims to quantitativelyprofile specific components present in several commercially-available allergen extract materials and strategize the engineering process using a recombinant component to adjust the responses of a patient panel to various materials, on the BioCLIA® 4G Allergy Assay.

Methods: Patient samples were purchased from plasma suppliers, and tested for peach (F95)-sIgE ImmunoCAP (Phadia, Sweden) reference values. Several commercially-available F95 materials and extractswere screened forrelative component contentby quantitative SDS-PAGE. Allergenic components, including Pru p 1, were identified using Western Blot on the WES platform (Protein Simple, San Jose, CA) with the patient panel. The patient panel was then tested for sIgE towards these raw materials and recombinant Pru p1 using the BioCLIA® 4G Allergy (HOB Biotech Group, China)Chemiluminescence Immuno Assay (CLIA) assays. Subsequently these extracts were supplemented with recombinant Pru p 1and the patient panel reactivity was re-assessed.**Results:** A panel of eighteen F95-positive sampleswasidentified. The sensitivity ofthis patient panel variedacross different F95 raw materials, ranging from 89% forVendor 1, 42% for Vendor 2and 53% for Vendor 3. WES positive signal resulted froman unidentified component of ~95 kDain Vendor 1 material, an unidentified component of ~66 kDain Vendor 2 material, and both ~66 kDa and ~95 kDa inVendor 3 material. All materials were essentially missing Pru p1.Eleven samples (61%) showed reactivity to rPrup1(rPru p 1-sIgE ≥ 0.35 IU/ml), with a response profile different from those of the three F95 materials. Adding defined ratios of rPru p1 increasedthe sensitivity of all three materials. **Conclusion:** This study has shown that raw material extracts are comprised of unique sIgE-binding components in varying concentrations leading to variable in vitro allergen-sIgE testing results, neither commutable across test methods nor consistent across lots of reagents. With modern analytical SDS-PAGEand capillary Western blot, raw materialcomponent ratioscan be identifiedin a patient-specific manner. Through guided adjustment of allergeniccomponentsin raw materials, test sensitivity can be greatly increased, particularly when important allergens are underrepresented or lacking.In summary, quantitative component composition information can be used to standardize allergen raw materials, engineer allergen-sIgE testing materials, and generate QC specifications to ensure lot-to-lot consistency.

A-359

A Sensitive Method for the Determination of Anti-CCP on the HOB 4G BioCLIA® 1200 Automated Immunoassay Analyzer

L. Cui, L. Liu, Z. Zhao, T. Zhao, X. Liao, X. Gen, J. Li, S. Yang, C. Zhou, C. Lee. *HOB Biotech Group, Suzhou, China*

Background: Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting between 0.5-1 % ofthe adult population. Patients with RA has been found to develop endogenous auto-antibodies to cycliccitrullinated peptides (anti-CCP), and these are used as a marker in the diagnosis of early RA. Since the first report in 1998 that antibodies present in blood and reactive with synthetic peptides containing the amino acid citrulline are highly specific for RA, the measurement of anti-CCP in patient serum has become important in the early and accurate diagnosis of this disease. Recently, the innovative HOB 4G BioCLIA® anti-CCP kit, coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system, has been launched for detection anti-CCP antibody in serum .

Methods: In this study, the analytical characteristics including limit of detection (LOD), precision (intra-assay & inter-assay), dilution linearity, and interference were evaluated by BioCLIA® anti-CCP kit according to the CLSI guidelines. 235 clinical samples with RA collected from Beijing Xiehe Hospital Rheumatism Immunity Branch were analyzed by both BioCLIA® and EURODIAGNOSTICA(ELISA) kits. Sensitivity, specificity and total agreement were analyzed between two compared assays. Lastly, anti-CCP titers were measured and analyzed with sera from Chinese patients with RA(N=165), rheumatic diseases other than RA (non-RA) (N=100), Systemic Lupus Erythematosus (SLE) (N=98), osteoarthritis (OA) (N=45), chronic inflammatory diseases (CID) (N=120) and healthy donors (N=150). At the same time,the detection rate was studied by both HOB BioCLIA®RF and HOB BioCLIA® CCP kits in the RA group (N=165). **Results:** The BioCLIA®anti-CCP kit performed good linearity ranging from 2~400RU/mL, and the LOD was 0.064RU/mL. In the precision testing, the CV% was 5.77% for intra-assay and 8.14% for inter-assay, respectively. Bilirubin(up to 20 mg/dL), hemoglobin (up to 150 mg/dL), and lipid (up to 1000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 2000 mg/dL) did not affect the detection of anti-CCP in serum. In a clinical evaluation, using 235 clinical samples with RA patients, we found the BioCLIA®has similar sensitivity 92.8%(116/125)with EURODIAGNOSTICA assay (ELISA),and a similar specificity of 91.8%(101/110). The total agreement of BioCLIA®& ELISA were 92.8%(217/235), respectively. The clinical sensitivity of RA was 70%(116/165) and the specificity for non-RA,SLE,OA,CID, and healthy donors were 97%(97/100), 97%(95/98), 98%(44/45), 98% (117/120), 98% (147/150). By comparison, the prevalence rate of anti-CCP and anti-RF antibodies in the RA group was 70%(116/165) and 54%(89/165), respectively.

Conclusion:

BioCLIA®anti-CCP kit is an innovative assay which exhibits fast and accurate analysis and demonstrates linearity in an extended analytical measuring range as well as good reproducibility. In the aspect of clinical comparison, the kit offered a better clinical relevance when compared with ELISA with a good total agreement of 92.8%. In conclusion, the BioCLIA®1200 anti-CCP kit is a sensitive and specific method in the detection of anti-CCP and valuable to aid other than anti-RF in the diagnostic process, treatment and monitoring of RA.

A-360

Comparison of the Agreement of 3 Serodiagnosis Algorithms for Syphilis in West China

D. Li, T. Wang, C. Tao. *West China Hosipital of SCU, Chengdu, China*

Background

The resurgence of syphilis in recent years has become a serious threat to the public health worldwide, and its diagnosis relies upon a combination of tests, including treponemal immunoassays (TT) and non-treponemal tests (NTT). The toluidine red unheated serum test (TRUST), T. *pallidum* particle agglutinationassay (TPPA) plus chemiluminescene immunoassay (CLIA) are employed in our hospital. There have not too many studies involving detailed analysis of the different algorithms for detecting syphilis.

Methods

Results of 4210 plasma sample simultaneously evaluated using the TRUST, TPPA, and CLIA were retrospectively collected study to analyze 3 syphilis testing algorithms: traditional algorithm, reverse algorithm, and the European Centre for Disease Prevention and Control (ECDC) algorithm (Fig 1). The kappa (κ) coefficient was used to compare the concordance between algorithms. The agreement of the results according to their κ values was categorized as near perfect (0.81-1.0), substantial (0.61-0.8), moderate (0.41-0.6), fair (0.21-0.4), slight (0-0.2), or poor (<0).

Results

Overall, 1477 subjects had TRUST+/TPPA+/CLIA+ results, and 527 subjects had TRUST-/TPPA-/CIA-results; 6 subjects were TRUST+/TPPA-. Among these 6 subjects, only 1 was CLIA+, which was discordant with the TPPA test result; These 5 TRUST+/TPPA-/CIA- cases were considered to have biological false positive reaction. 6 subjects were TRUST-/TPPA+/CLIA- without the presence of the prozone phenomenon may be considered to acute or early infection. 220 TRUST-/CLIA+/TPPA cases indicated the high sensitivity of CLIA assay. The overall percentage of agreement and κ value between the reverse algorithm started by TPPA and the ECDC algorithms were 99.0% and 0.968, respectively. The overall percentage of agreement and κ value between the reverse algorithm started by CLIA and the ECDC algorithms were 89.5% and 0.655.

Conclusions

It is recommended to use of the ECDC algorithm started by CLIA, complemented with a NTT test to determining serological activity and the effect of syphilis treatment.

Key words: syphilis; algorithm; diagnosis

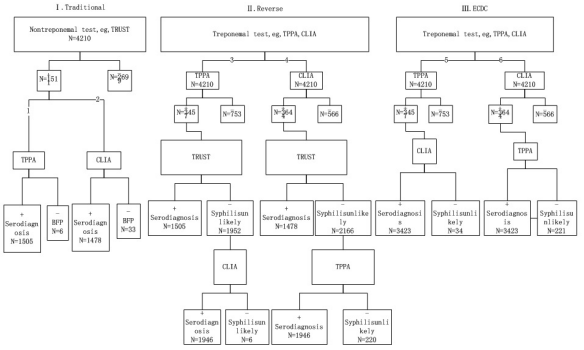


Fig1. Syphilis testing algorithms. Abbreviations: BFP, biological false positive; CIA, chemiluminescence immunoassay; ECDC, European Centre for Disease Prevention and Control; EIA, enzyme immunoassay; TRUST, toluized red unheated serum test; TPPA, Treponema pallidum particle agglutination.

A-361

Performance Evaluation of the ADVIA Centaur Interleukin-6 (IL-6) Assay

S. Poynton[1], C. Grieve[1], N. McIntosh[1], A. Suttie[1], P. Kelly[1], D. Hovanec-Burns[2]. *[1]Axis-Shield Diagnostics, Dundee, United Kingdom, [2]Siemens Healthcare, Tarrytown, NY*

Background: Interleukin-6 (IL-6) is a pleiotropic cytokine with a broad range of biological activities. IL-6 production is rapidly induced in the course of acute inflammatory reactions associated with a number of clinical situations, including infection and may be used as an aid in the study of inflammatory diseases. The ADVIA Centaur Interleukin-6 (IL-6) assay* for the quantitative measurement of IL-6 in human serum and plasma on the ADVIA Centaur® Immunoassay Systems is under development by Axis-Shield Diagnostics Inc. for Siemens Healthcare Diagnostics. The objective of this study was to evaluate assay performance for precision, linearity, limit of quantitation (LoQ), interference and cross-reactivity, and method comparison to the IMMULITE® 2000 IL-6 assay† (Siemens Healthcare Diagnostics Inc.).

Method: The ADVIA Centaur IL-6 assay is a sandwich immunoassay that employs direct chemiluminescent technology. IL-6 is bound to mouse monoclonal anti-IL-6 antibody-coated magnetic particles and is then detected by an acridinium ester-labelled anti-IL-6 mouse monoclonal antibody. Following incubation, a wash step, and magnetic separation, acid and base reagents are added to the reaction mixture and the resulting chemiluminescence is measured. The method comparison study was performed per CLSI EP-09-A3 using 276 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A3. Linearity and LoQ studies followed CLSI EP17-A2 and EP06-A, respectively. Per CLSI EP07-A2, the assay was tested for interference from hemoglobin, bilirubin (conjugated and unconjugated), triglyceride, biotin, cholesterol, immunoglobulin G, total protein, rheumatoid factor, and lipemia. Also per CLSI EP07-A2, the assay was tested for cross-reactivity with soluble IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-8, TNF-α, TNF-β, IFY-γ, IFN-α, IFN-β, and IL-6 receptors.

Results: Observed reportable range of the assay was up to 5000 pg/mL without dilution and up to 50,000 pg/mL with automated or manual 1:10 dilution. The assay's observed linearity was up to 5000 pg/mL. The limit of quantitation was observed at 1.8 pg/mL, with a total error of 35%. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 4.0–6.8%. Passing-Bablok method comparison of the assay to the IMMULITE 2000 IL-6 assay returned a slope of 1.04 and an intercept of -1.31pg/mL, with a Pearson correlation r value of 0.98. The assay demonstrated no interference and no cross-reactivity with the tested analytes.

Conclusions: The feasibility of an automated IL-6 assay on the Siemens ADVIA Centaur XP Immunoassay System has been assessed. Preliminary performance results show an accurate and precise assay for the measurement of IL-6.

*Under development. The performance characteristics of this product have not been established. Not available for sale, and its future availability cannot be guaranteed.

† Not available for sale in US

A-362

Evaluation of the Analytical Performance of Anti-Mitochondrial IgG Antibodies on the HOB BioCLIA® 1200 Automated Immunoassay Analyzer

L. Liu, Z. Zhao, L. Cui, T. Zhao, X. Liao, X. Gen, J. Li, S. Yang, C. Zhou, C. Lee. *HOB Biotech Group, Suzhou, China*

Background: Anti-mitochondrial IgG antibodies is one of the primary auto-antibodies present in patients with Primary Biliary Cirrhosis (PBC) and in vitro detection of Anti-Mitochondrial IgG antibody is the classic serological marker of PBC. Early studies described 9 subtypes of mitochondrial antigens, termed M1-M9. The major autoantigens targeted by PBC patient sera recognize the M2 antigen fraction. The primary components of the M2 antigen were found to be members of the dehydrogenase complex, the specific antigens were identified as the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), 2-oxo-glutarate dehydrogenase complex (OGDC-E2). branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2). Recently, the innovative HOB BioCLIA® AMA-M2 kit, coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system has been launched. **Methods:** In this study, the analytical characteristics including limit of detection (LOD), precision (intra-assay & inter-assay), dilution linearity, and interference were evaluated by HOB BioCLIA® AMA-M2 kit according to the CLSI guidelines. Furthermore, 300 clinical samples with indirect immunoflurescence assay (IFA) results, were analyzed by both BioCLIA® and ELISA (from an international renowned manufacture). Sensitivity, specificity and total agreement of the compared assays were analyzed. Lastly, a total of 340 clinically characterized samples were used to study clinical sensitivity and specificity, specifically 100 patients for PBC, 50 patients for Systemic Lupus Erythematosus (SLE), 60 patients for Rheumatoid Arthritis (RA), 30 patients for virus hepatitis, 100 samples for healthy donors. **Results:** The BioCLIA® AMA-M2 kit performed good linearity ranging from 2-400 RU/mL, and the LOD was 0.15 RU/mL. In the precision testing, the CV% was 4.38% for intra-assay and 5.05% for inter-assay, respectively. Bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL) did not affect the detection of anti-Mitochondrial antibody IgG in serum. In a clinical evaluation, using 300 clinical samples with IFA assay results, we found the BioCLIA® has higher sensitivity 97.0% (97/100) than ELISA results 90% (90/100), but with a similar specificity of 97%. The total agreement of BioCLIA® & ELISA compared to IFA assay were 97% (291/300) & 94% (284/300), respectively. From the clinical study in Chinese patients, the positive rate showed on HOB BioCLIA® AMA-M2 kit in PBC, SLE, RA, virus hepatitis and healthy donors were 92% (92/100), 8% (4/50), 1.6% (1/60), 0% (0/30) and 1% (1/100), respectively. **Conclusion:**

BioCLIA® AMA-M2 kit is an innovative semi-quantitative assay which exhibits fast and accurate analysis and demonstrates linearity in an extended analytical measuring range as well as good reproducibility. In the aspect of clinical comparison, the kit offered a better clinical relevance when compared with ELISA, and a good agreement (positive agreement=97%, negative agreement=97% and total agreement = 97%) with IFA assay, which is considered as the gold standard method. In conclusion, the BioCLIA® AMA-M2 assay is a sensitive and specific method, which could serve as a promising and fully automated alternative for IFA assay in the detection of anti-mitochondrial IgG antibodies and valuable to aid in the diagnosis of PBC.

A-363

Sensitive and Quantitative Methods of Anti-cardiolipin Antibodies Determinations with HOB 4G BioCLIA®Technologies

L. Liu, T. Zhao, L. Cui, Z. Zhao, X. Liao, X. Gen, J. Li, S. Yang, C. Zhou, C. Lee. *HOB Biotech Group, Suzhou, China*

Background: Anti-cardiolipin antibodies (aCL), against anionic phospholipids or protein phospholipid complexes, are strongly associated with venous, arterial thrombosis and obstetrical complications. These symptoms were defined as anti-phospholipid syndrome (APS) by Harris in 1987. High levels of aCL-IgG are known to be most diagnostically valuable for APS. aCL-IgA and aCL-IgM, relative to hemolytic anemia, also exist in APS patients. Four types of BioCLIA® aCL kits were launched, including cardiolipin IgG, IgA, IgM, and cardiolipin screening kits. The BioCLIA® aCL kits were more accurate, sensitive, quantitative and automated than traditional ELISA or LIA kits.

Methods: The analytical performancesincluding limit of detection (LOD), dilution linearity, precision (intra-assay & inter-assay), and interference were evaluated by HOB BioCLIA® aCL kits, according to CLSI guidelines. Method comparison was

conducted using300clinical samples by both HOB BioCLIA® kits and ELISA kits (from international renowned manufactures). Clinical sensitivity and specificity were analyzed from various disease patients including 88 APS patient samples, 102 systemic lupus erythematosus (SLE) patient samples,105 rheumatoid arthritis (RA) patient samples,10 syphilis patient samples and 100 normal healthy donors.All samples mentioned were collected from major Chinese hospitals. **Results:** The LOD of the cardiolipin IgG, IgA, IgM, and cardiolipin screen kits were 0.41RU/mL, 0.13RU/mL, 0.06RU/mL, 0.06RU/mL, respectively. The linearity range of the four types of aCL kits were all ranging from 2 to 400RU/mL, and the relative coefficients were all above 0.99. In the precision testing of the four types of aCL kits, the CV% of intra-assay and inter-assay were all below 10%. Bilirubin, hemoglobin, and lipid, RF and HAMA proved to be no influence on the detection of aCL-IgG, aCL-IgA or aCL-IgM in serum. In the comparison with ELISA kits (EUROIMMUN or AESKULISA ELISA kits), the negative agreement of cardiolipin IgG kit, cardiolipin IgA kit, cardiolipin IgM kit, and cardiolipin screening kit were all above 90%, and their positive agreements were 77.0% (57/74), 86.1% (31/36), 80.8% (42/52), 90.9% (110/121), respectively. As for clinical sensitivity and specificity,it indicated 52 positive aCL-IgG (59.1%), 33 positive aCL-IgM (37.5%), 25 positive aCL-IgA (28.4%)and 72 positive aCL-IgA/IgG/IgM (81.8%) of 88 APS samples. 11 positive aCL-IgG (10.8%), 9 positive aCL-IgM (8.8%), 7 positive aCL-IgA (6.9%)and 13 positive aCL-IgA/IgG/IgM (12.7%) of 102 SLE samples were also detected. And 3 positive aCL-IgG (2.9%), 4 positive aCL-IgM (3.8%), 2 positive aCL-IgA (1.9%)and 5 positive aCL-IgA/IgG/IgM (4.8%) of 105 RA samples were detected.It showed all negative in syphilis samples or normalhealthy samples by HOB BioCLIA® 4G aCL kits. **Conclusion:** Coupled with the fullyautomated BioCLIA® 1200 ImmunoAssay Analyzer, the HOB BioCLIA®4G aCL kitsshowed faster and more accurate results with extended working ranges and good reproducibility. It consists of 3 individual Assay (aCL-IgA, aCL-IgG, aCL-IgM) and a screening assay (aCL-IgA/IgG/IgM) with comparable results with ELISA for detection of aCL-IgA, aCL-IgG, aCL-IgM&aCL-IgA/IgG/IgM. These clinical trials indicate that HOB BioCLIA®4GaCL kits will provide better diagnosis and treatment for patients with APS.

A-364

A Novel Magnetic Bead Chemiluminescence Immunoassay (CLIA) for Quantitative Determination of Autoantibodies to Glutamic Acid Decarboxylase (GAD65) and IA-2 in Serum

C. Zhou, F. Sun, W. Wu, S. Cao, L. Liu, C. Lee. *HOB Biotech Group, Suzhou, China*

Background: The type 1 diabetes mellitus (T1DM) is characterized by insufficient production of insulin by the pancreas being called autoimmune destruction, affecting mainly children, adolescents and young adults. The autoantibodies to pancreatic beta cell antigens are important markers of T1DM. Presently there are 5 major autoantibodies that are used to define risk for T1DM: GADA, IA-2A, ICA, IAA and ZnT8A. The novel BioCLIA® GAD and IA-2 kits coupling with the fully automated, random-access BioCLIA® 1200 system aimed to measure the GAD and IA-2 antibodies in serum. **Methods:** The analytical performances including dilution linearity, limit of detection (LOD), precision (intra-assay & inter-assay), and interference were evaluated by BioCLIA® GAD & IA-2 kits according to CLSI guidelines. 100 clinical samples with Euroimmun ELISA results were also tested by BioCLIA® GAD and IA-2 kits. Meanwhile, total of 148 disease confirmed clinical samples (from a major hospital in China) were tested for GAD and IA-2 autoantibodies. Out of these samples, 38 were from the newly diagnosed T1DM patients, 50 were T2DM and the remaining 60 samples were confirmed negative from diabetes. **Results:** The BioCLIA® GAD and IA-2 kits both performed outstanding dilution linearity ranging from 2-2000 and 5-4000 IU/mL, respectively. The LOD was 0.0683 IU/mL for GAD kit and 0.045 IU/mL for IA-2 kit. In the precision study, the intra-assay CV were 2.59% and 2.2% and inter-assay CV were 6.30% and 5.20% for GAD and IA-2 kits respectively. Furthermore, the two kits showed no significant influence by bilirubin (up to 40 mg/dL), hemoglobin (up to 500 mg/dL), lipid (up to 3000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL). Compared to the ELISA, we found the sensitivity of the GAD and IA-2 kits were 84.0% (42/50) and 90.0% (45/50) and the specificity were both high upto 98% (49/50), respectively. Furthermore, in the evaluation result from 148 clinical samples, the sensitivity of GAD kits was to be 71% (27/38) and IA-2 kits was 76.3% (29/38). Notably the sensitivity was improved to 92.1% (35/38) when we simultaneously use these two kits for the measurement of GADA and IA-2A in the T1DM patient serum. In the same time the specificity of GAD and IA-2 kits were both high upto 98.3% (59/60) and 98% (49/50), respectively. Similarly, the specificity were 96.7% (58/60) and 98% (49/50) when we simultaneously use these two kits to measure the 60 healthy samples and 50 T2DM samples.

Conclusion: The BioCLIA® GAD and IA-2 kits exhibit fast and accurate analysis with an extended dynamic range as well as good reproducibility. It could serve as a promising and environmental-friendly alternative for IFA and ELISA assays in the detection of T1DM autoantibodies and would be a well-deserved choice in the diagnosis of T1DM.

A-366

Performance Evaluation of Der p1 and Der p2 specific IgE in Chinese House Dust Mite Allergy Patients on the HOB BioCLIA® Analyzer

L. Qian, X. He, P. Qian, P. Qian, Y. Wang, Y. Liu, G. Hu, Y. Tao, C. Lee. *HOB Biotech Group, Suzhou, China*

Background: The incidence of allergic diseases steadily increases year after year. Component-Resolved Diagnostics (CRD) utilize purified native or recombinant allergens to detect sensitization of patients to individual allergen molecules and have become of growing importance in clinical investigation of IgE-mediated allergies. The CRD could decrease the need for provocation testing and may also improve the specificity of allergen-specific immunotherapy. House dust mites (HDM) are among the most important allergen sources in the world.*Dermatophagoides pteronyssinus* and *Dermatophagpides farina* are the primary house dust mite species, while Der p 1 and Der p 2 represent the *D pteronyssinus* allergens with greatest clinical significance.

Methods: In this study, the analytical performance of BioCLIA® sIgE-Der p 1 and Der p 2Assays were evaluated on the fully automated, random-access BioCLIA® chemiluminescence analyzer, according to the CLSI I/LA20-A2 guideline. A total of 100 serum samples were collected from HDM allergy patients at local hospitals in China. Specific IgE to Der p 1 and Der p 2 were evaluated by BioCLIA® Allergy sIgE Assays (HOB Biotech, China) and ImmunoCAP (Phadia AB, Sweden).

Results: The BioCLIA® sIgE -Der p 1 and Der p 2 Assays showed exclusive reactivity to human IgE and performed with excellent linearity ranging from 0.1-100 IU/mL. The LoD of BioCLIA® sIgE - Der p 1 and Der p 2 Assays were 0.05 and 0.06 IU/mL. Good precision were observed with the intra-assay CV 3.1% and 2.2%, the inter-assay CV 6.2% and 5.6% for Der p 1 and Der p 2. Spiking recovery test showed the accuracy of Der p1 and Der p were 100.2%-103.8%, 97.9%-99.8%. Bilirubin (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), and lipid (up to 2000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL) did not affect the detection of the specific IgE in serum. Among all of the 100 HDM allergy patients, the sensitivity of HOB BioCLIA® Der p 1- and Der p 2performed against D1 samples were 69.8% and 72.3 %, respectively. HOB BioCLIA® Der p 1- and Der p 2-sIgE Assaysalso agree well with Phadia ImmunoCAP Der p1- and Der p 2-sIgE Assays, with positive agreements at95.2% and 97.2%, total agreementsat 96.8% and 98.4%, respectively. Regression analysis between HOB BioCLIA®and ImmunoCAP assays, the Der p1 was $Y=0.7466X+3.6787$, $R^2=0.7082$, and Der p 2 was $Y=0.6405X+3.9165$, $R^2=0.6422$.

Conclusion:

The innovative HOB BioCLIA® systemoffers well performing allergen-sIgE assays, including Der p 1- and Der p 2-sIgE assays on BioCLIA®, that are linear, precise, repeatable, reproducible,with extended dynamic ranges. Der p 1 and Der p 2 are the dominant allergens that elicitallergy in HDM allergic patients. BioCLIA®Der p 1- and Der p 2-sIgE Assays provide a promising diagnostic tool in the diagnosis of *D. pteronyssinus* sensitization and guidance for allergen-specific immunotherapy in HDM allergic patients.

A-367

Analytical and Clinical Performance of the Anti-TPO and Anti-TG Kits on the BioCLIA®1200 Automated Chemiluminescence Immunoassay Analyzer

Y. Li, S. Xu, C. Zhou, J. Gao, R. Wu, C. Lee. *HOB Biotech Group, Suzhou, China*

Background: The major thyroid autoimmune diseases are theHashimoto's thyroiditis and Graves' disease.Both thyroperoxidase (TPO) and Thyroglobulin (TG) are potentially autoantigenic. The anti-TPO is detected in most of Hashimoto's thyroiditis and Graves' disease samples. High levels antibodies of Anti-TPO and anti-TG, in the context of the clinical presentation of hypothyroidism deliver a much greater value to diagnosis of Hasimoto's disease. The BioCLIA®anti-TPO and anti-TG kits are designed for the specific, quantitative detection of anti-TPO or anti-TG in serum. BioCLIA®1200 chemiluminescent immunosassay system is a random-access, high-throughput and continuous automated platform.**Methods:** In our study, the analytical performances of BioCLIA® anti-TPO and anti-TG kits including the limit of

detection(LOD), intra-assay,inter-assayand accuracystudies were evaluatedaccording to CLSI guidelines. Total of 625 clinical samplescollected from major Chinese hospitals were analyzed and compared with Beckman Coulter DxI 800 assays. The discordant samples were re-analyzed by the same kits from Roche Cobas® and Abbott Architect® Systems. **Results:** The LOD of anti-TPO & anti-TG were 0.037 IU/mL and 0.305 IU/mL. The linear range of the anti-TPO & anti-TG wereestablished as 0.25-1000 IU/mLand 1.5-2500 IU/mL. Assay precision studies demonstrated acceptable CV% of <5.1% &<8.9% for intra-assay and inter-assay for anti-TPO and anti-TG. The anti-TPO assay was traceable to the WHO International Standard; NIBSC Code: 66/387, while the anti-TG assay was traceable to the WHO International Standard; NIBSC Code: 65/093.Total of 625clinical samples were compared between Beckman Coulter DXI-800 assays and HOB BioCLIA® assays. The regression analysis for anti-TPO with Passing-Bablok regression fit of HOB BioCLIA = 0.9982 Beckman Coulter DXI800 +2.72 (r=0.994).The positive agreement for anti-TPO and anti-TG were 97.6% (359/368) and 98.1% (362/369),the negative agreement for anti-TPO and anti-TG were 99.2%(255/257) and 99.6%(255/256). The total agreement for anti-TPO and anti-TG were 98.2% (614/625) and 98.7% (617/625). The discordant samples were confirmed by Roche's Cobas® and Abbott's Architect® systems to resolve their differences.**Conclusion:** The BioCLIA ®anti-TPO & anti-TG kits perform an extended working range and good precision &reproducibility. Excellent agreements were observed between HOB BioCLIA®anti-TPO & anti-TG and Beckman Coulter DXI-800 assays. The HOB BioCLIA ®anti-TPO & anti-TG kits provide the fast & accurate detection of anti-TPO & anti-TG in serum and can be used as valuable aid in diagnostic process, treatment of autoimmune thyroid diseases.

A-369

Measurement Limits on BioTek ELx800 ELISA Plate Reader for Biomarker Analysis

S. Narla[1], I. Patzer[1], J. Bailey[1], J. Emerson[1], R. Philippe[2], D. Cavard[2], F. Martine[2], P. Oefinger[1]. *[1]Covance Central Laboratories, Indianapolis, IN, [2]Covance Central Laboratories, Geneva, Switzerland*

Background: The BioTek ELx800 is a single-channel reader-assay system, designed to automatically perform endpoint analysis for ELISA-based applications. The current acceptance criteria for Optical Density (OD) is up to 2.75 at 450 nm which was determined by performing an absorbance plate test, but the readers are capable of reading up to OD 4.00. This test uses BioTek's Absorbance Test Plate (Part: 7260522, BioTeK) to confirm the Mechanical Alignment, Accuracy, Linearity, and Repeatability of the ELx800 at 450 nm. The Absorbance Plate Test compares the reader's optical density measurements and mechanical alignment to NIST-traceable values. However, often the OD's encountered are higher than 2.75 for many newly developed biomarker ELISA kits, leading to more failed runs and/or increasing more repeat runs with sample dilutions.

Methods: The linearity and % CV are considered as acceptable assessments of accuracy. A liquid test using a dye solution was performed to evaluate the accuracy of the plate reader at OD > 2.75. To perform the liquid test at 450 nm, a stock liquid test solution (QC Check #1, PN 7120782, BioTek Instruments, Inc.) was diluted to obtain an OD reading as close as possible to 4.00. This solution was taken as 100% solution and was diluted in 5% increments up to 5% with DI water. These samples (200µL volume) were added to an ELISA plate in duplicate wells; whereupon the plate was read at 450 nm for 5 times to assess repeatability. The mean OD of duplicate wells was calculated for each dilution. Regression analysis was performed to identify the OD at which the results are linear. An R^2 value greater ≥ 0.990 is considered acceptable for linearity; while the %CV ≤1.5% is considered acceptable for repeatability. Reading of the experimental plate was repeated on three different plate readers across our two laboratory institutions.

Results: All readers yielded linear results with ODs ≥ 3.30 for a linear and log-log linear curve fit with an R^2 >0.99. Additionally, a 4-parametic logistic (4-PL), 5-parametic logistic (5-PL), spline and polynomial^2 curves were assessed by the ELx800 software (Gen 5 Secure 2.00.18) on one reader at each laboratory. The readers yielded linear results with ODs ≥ 3.50 for all these curves with R2 of >0.99. Also, all readers yielded acceptable repeatability with %CV ≤1.5%.

Conclusion: At an absorbance of 450 nm, ELx800 plate readers yielded linear and accurate results with OD of 3.30 for linear and log-log linear curves and up to 3.50 OD for 4PL, 5PL, spline and polynomial^2 curves. Therefore, the achieved ODs can be used as upper limit for the respective curves. This extension of ODs is beneficial by reducing failed and/or repeat runs, thus saving samples, labor time and expenses.

A-370

Validation of EUROIMMUN ELISA Kit for Neurogranin TruncP75 Measurement in Cerebrospinal Fluid (CSF)

S. Narla[1], J. Emerson[1], T. Allen[1], P. Oefinger[1], H. Vanderstichele[2]. *[1]Covance Central Laboratories, Indianapolis, IN, [2]ADx NeuroSceinces, Gent, Belgium*

Background: Alzheimer's disease (AD) is the most common neurodegenerative disorder and is a major health problem among older people. AD can be characterized by pathological hallmarks such as Amyloid-β containing neuritic plaques and neurofibrillary tangles composed of hyper-phosphorylated tau proteins in CSF. Synaptic dysfunction and loss are directly linked to memory disturbances and other cognitive symptoms that are present at the early stages of AD. The synaptic loss occurs early and it correlates with cognitive deficits in patients with AD. Therefore, the measurement of synapse proteins in CSF could be useful for studies of disease mechanism, to improve tools for early diagnosis and prognosis, and to monitor drug effects on synaptic degeneration in clinical trials of disease modifying therapies for AD. The postsynaptic protein Neurogranin in CSF has been reported to increase in AD, including pre-dementia stage of disease, making it a promising CSF biomarker.

Methods: The EUROIMMUN ELISA test kit (EQ6551-9601-L) is designed for the quantitative determination of Neurogranin truncated at P75 in CSF samples. In the first analysis step, samples are incubated with biotinylated monoclonal anti-Neurogranin antibody, followed by addition to microplate wells coated with monoclonal antibodies specific for human Neurogranin truncated at P75. In this process, truncated Neurogranin is bound in a complex. In a second incubation, the biotin binds to streptavidin peroxidase conjugate. Incubation of the complex with substrate and chromogen promotes a color reaction. The color intensity is proportional to the truncated Neurogranin concentration in the sample.

Results: Linearity was established by using CSF spiked with 8 concentration levels of Neurogranin, each level run 3 times. The method was linear up to 1000.0 pg/mL. The Lower Limit of Quantification (LLOQ) was established using CSF spiked with 5 concentration levels of Neurogranin tested in replicates of 40 over 5 days, LLOQ was set at 50.0 pg/mL with a %CV of 17.0. The within-run precision was performed using 2 levels of QC that were run 20 times on single plate; the average %CV is ≤ 5%. For between-run precision the 2 levels of QC were run over 10 different runs over 10 days, the average CV is ≤ 6.6 %. Accuracy was accessed based on the recovery of CSF spiked with 6 concentration levels of Neurogranin, with an average recovery of 94%. The maximum manual dilution was verified up to 1:8 with a recovery of 97% at 1:8 times dilution, extending the upper limit of quantification (ULOQ) to 8000.0 pg/mL. To analyze the Length of Run, 2 kit QC samples were assayed across the plate over three runs, yielded no significant difference observed with results generated across the plate.

Conclusion: The EUROIMMUN Neurogranin ELISA kit was successfully validated as per the CLSI guidelines. The assay kit is suitable to perform testing on CSF samples to assess the concentration of Neurogranin.

A-371

Determination of Infliximab Drug Levels by the ALPCO Infliximab Drug Level ELISA and Method Comparison to LC/MS-MS

B. Bell[1], R. Pollok[1], M. A. Willrich[2], M. Snyder[2], L. A. Brunelle[1]. *[1]ALPCO, Salem, NH, [2]Mayo Clinic, Rochester, MN*

Background: Infliximab is a biologic therapy widely used to treat inflammatory bowel disease (IBD). Efficacy of this therapy is highly dependent upon maintaining the appropriate levels of drug between treatment sessions. Several infliximab immunoassays exist which allow physicians to monitor drug level status in their patients and tailor treatment regimens accordingly, however method standardization across the industry has not been established to date. This study is aimed to investigate method comparability of the ALPCO® Infliximab Drug Level ELISA to tandem mass spectrometry (LC/MS-MS).

Methods: Serum samples (n=30) from patients undergoing infliximab treatment were classified as containing low (n=10), medium (n=10) or high (n=10) levels of infliximab determined using an LC/MS-MS method (API 5000, AB SCIEX) at the Mayo Clinic, Rochester, MN. Frozen aliquots (-80°C) were blinded then subsequently analyzed by the ALPCO Infliximab Drug Level ELISA according to the instructions for use. Experiments were performed manually for the ALPCO assay; results were read on the VERSAmax plate reader (Molecular Devices) and data analysis was performed using SoftMax® Pro GxP software. For the ALPCO assay, intra- (n=24) and inter-assay (n=10) precision was performed according to CLSI Guidelines. Limit of

quantitation (LOQ) of the ALPCO assay was determined according to CLSI Guideline EP17-A2 with a specified accuracy goal of a coefficient of variation (CV) less than or equal to 20%. Dilutional linearity was also assessed.

Results: A quantitative comparison between the ALPCO assay and the LC/MS-MS assay focused on values in the clinically relevant range (up to 20 µg/mL) with values below the LOQ (ALPCO = 0.7 µg/mL and LC-MS/MS = 1.0 µg/mL) normalized to their respective absolute LOQ values. The comparison demonstrated a good correlation between the two methods with a slope of 0.81 and R^2 of 0.947. The ALPCO assay demonstrated precision values with CVs less than or equal to 11% and dilutional linearity studies showed a slope of 1.00 and R^2 of 0.989.

Conclusion: This study demonstrates that the ALPCO immunoassay, currently available in this field, provides a reliable means by which to quantitate serum levels of infliximab as an alternative to mass spectrometry. Although some level of bias exists between the ELISA when compared to an established LC/MS-MS method, the clinical information provided by both methodologies are similar. While these methods provide comparable results, industry wide standardization would benefit all methods in the field.

A-372

Assessing the Laboratory Protocol for the Hevylite® Assay on a Multiple Myeloma Patient Cohort

S. M. Kenyon, M. C. Kohlhagen, L. Tostrud, M. R. Snyder, J. A. Katzmann, D. L. Murray, M. A. Willrich. *Mayo Clinic, Rochester, MN*

Background: The Hevylite® assay (HLC) quantifies intact immunoglobulin (Ig) heavy and light chain pairs and is complementary to serum protein electrophoresis (SPEP) for monitoring patients with multiple myeloma (MM). An abnormal Igκ/Igλ ratio suggests a monoclonal immunoglobulin. The HLC assay may overcome some of the known limitations of SPEP, including difficulty detecting monoclonal proteins co-migrating with other serum components. At our institution, HLC is performed in conjunction with nephelometric total Ig measurements to evaluate antigen excess. An expected recovery (sum of Igκ + Igλ to total Ig) of 80-120% was established, analogous to the one used for IgG subclasses measurement. However, empirical observations suggest HLC recoveries from MM patient samples often fall outside this range, resulting in additional dilution steps/repeats to confirm results.

Objective: The objective of this study was to assess the laboratory protocol for measuring HLC that compares percent recoveries between HLC and total IgG measurements on a MM patient cohort.

Methods: Diagnostic serum samples from patients with untreated IgGκ (n=241), IgGλ (n=124), IgAκ (n=99), and IgAλ (n=54) MM were analyzed for SPEP (Helena, Beaumont, TX), immunofixation (Sebia, Lisses, FR), and total Ig (Siemens, Munich, DE) and HLC (Binding Site, Birmingham, UK) on the BNII System (Siemens). Involved HLC (iHLC) concentrations and Igκ/Igλ ratios were calculated. The clinical sensitivity of HLC, as determined by elevated Igκ/Igλ ratios for IgGκ and IgAκ and low Igκ/Igλ for IgGλ and IgAλ, was reviewed for each HLC pair. Percent recoveries (median and range) and the number of measurements outside the 80-120% recovery range were calculated. For all samples outside the recovery range, iHLC concentrations and Igκ/Igλ ratios were assessed to determine if they were outside the published reference intervals. Potential antigen excess misses were defined as <50% recovery, combined with assessment of Igκ/Igλ ratio and iHLC.

Results: Of the 518 MM patient samples, 509 (98%) had a measurable M-protein on SPEP. The clinical sensitivity was determined to be 97% for IgGκ, 98% for IgGλ, 97% for IgAκ, and 96% for IgAλ, with 503 samples (97%) having an abnormal Igκ/Igλ ratio, and 494 samples with elevated iHLC concentrations. The median recovery was 82% for IgGκ (range: 35-136%), 80% for IgGλ (50-140%), 130% for IgAκ (4-246%), and 110% for IgAλ (41-164%). A total of 254 measurements (49%) were outside the 80-120% recovery range (104 IgGκ, 64 IgGλ, 67 IgAκ, 19 IgAλ). 170 measurements were assessed for antigen excess misses, with only one, the IgAκ measurement with 4% recovery, showing evidence of true antigen excess. For measurements with recoveries >120% (84), all samples had elevated iHLC concentrations and/or abnormal Igκ/Igλ ratios.

Conclusions: Of the 518 MM samples analyzed, nearly half fell outside the 80-120% recovery range, resulting in additional dilutions and replicate measurements. Despite repeating 254 measurements, only one demonstrated evidence of antigen excess miss, indicating repeating all measurements outside the 80-120% recovery range is not justified for HLC. While discontinuing the practice of %recovery comparison entirely seems daring, an option to improve lab workflow could include only repeating measurements that recover <10%, which would reduce the repeats to 2%.

A-373

Validation of a Non-FDA Approved Specific IgE Allergen (RF345 Macadamia Nut) by Fluoroenzymeimmunoassay.

J. C. Harrington, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: Quantitative detection of circulating IgE antibodies can be used to evaluate patient allergies and determine sensitization to a specific allergen. There is a direct relationship between the concentration of IgE antibodies and the probability of allergic symptoms which include itchy eyes, eczema, rhinitis, bronchoconstriction, vomiting, diarrhea, and anaphylaxis. Here we report the performance characterization of an allergy assay for macadamia nut by fluoroenzymeimmunoassay.

Method: Validation testing was performed on the Phadia Thermo Scientific ImmunoCAP 1000 (Phadia US Inc., Portage, MI, USA) using leftover patient serum samples in our laboratory. The performance validation included linearity, precision, method comparison, interference, sample stability, and reference range. The data was analyzed using EP Evaluator Version 10 (Data Innovations LLC, Burlington, VT, USA).

Results: Assay linearity was assessed by serial diluting a high patient specimen with the sample diluent included in the reagent package and assaying the resulting specimens in triplicate. The validated linear range was 0.35-100.00 kUA/L with a maximum dilution factor of 200. Within-day precision was evaluated by assaying a low patient pool and a high patient pool 10 times in a batch, and was found to be 4.5% and 4.6%, respectively. The between-day precision was assessed by analyzing the same pools twice a day for 10 days, and found to be 5.6% CV (low pool) and 5.3% CV (high pool). The method was compared to a previously vetted Phadia Thermo Scientific ImmunoCAP 1000 using leftover patient specimens (n=40). The Deming regression showed an R of 0.9999, an intercept of 0.034, a slope of 1.040, and a mean difference of 4.4%. There was no significant interference by lipemia, hemolysis, icterus, and uremia. The analyte was found to be stable in serum for 48 hours ambient (18-32°C), 7 days refrigerated (2-8°C), and 14 days frozen (-20°C). The reference range was verified at <0.35 kUA/L using 43 patient samples with normal total IgE and no indication of macadamia nut in the patient charts.

Conclusion: The method performance characteristics of the non-FDA approved specific IgE allergen RF345 Macadamia Nut assay were consistent with Phadia specific IgE allergen assays that are approved for in vitro diagnostics by FDA.

A-374

Performance Evaluation of the ADVIA Centaur aCCP Assay

G. Campbell[1], E. Bull[1], D. Hovanec-Burns[2]. *[1]Axis-Shield Diagnostics, Dundee, United Kingdom, [2]Siemens Healthcare, Tarrytown, NY*

Background: Anti-cyclic citrullinated peptide IgG (aCCP) is a specific marker for rheumatoid arthritis (RA), a disease that affects 1% of the population worldwide. Studies show that the presence of anti-CCP IgG can be detected in the early stages of RA and is indicative of a more-progressive form of the disease. Measurement of aCCP, in conjunction with other laboratory findings and clinical assessments, may be used as an aid in the diagnosis of RA. Autoantibody levels represent one parameter in a multi-criteria diagnostic process encompassing both clinical and laboratory-based assessments. The ADVIA Centaur® aCCP assay (Siemens Healthcare Diagnostics Inc.) is intended for the semi-quantitative determination of the IgG class of autoantibodies specific to cyclic citrullinated peptide in human serum or plasma (K2-EDTA and lithium heparin) using the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate assay performance for precision, linearity, limit of detection (LoD), interference and cross-reactivity, and method comparison to the ARCHITECT anti-CCP assay (Abbott).

Method: The ADVIA Centaur aCCP assay is a fully automated, two-step immunoassay using chemiluminescent technology. The assay utilizes an acridinium ester-labelled anti-human IgG as the Lite Reagent. The solid phase consists of biotinylated CCP coupled to streptavidin, which is then coated onto magnetic latex microparticles. The method comparison study was performed per CLSI EP-09-A3 using 253 patient samples. A precision study was executed over 20 days using serum samples according to CLSI EP5-A2. Linearity and LoD studies followed CLSI EP17-A2. Per CLSI EP07-A2, the assay was tested for interference from hemoglobin, bilirubin (conjugated and unconjugated), triglyceride, biotin, total protein, rheumatoid factor, lipemia, and caprine IgG. Also per CLSI EP07-A2, the assay was tested for cross-reactivity on 22 subgroups of non-RA subjects (n = 460) with potentially cross-reacting conditions and disease states where other autoantibodies may be present in the subject samples.

Results: The ADVIA Centaur aCCP assay measured anti-CCP IgG concentrations from 0.40 to 200.00 U/mL, with LoD determined to be 0.40 U/mL. The assay was determined to be linear from 0.40 to 200.00 U/mL. In a 20-day precision study, the assay demonstrated within-laboratory imprecision of 3.0–4.3%. The diagnostic concordance between the ADVIA Centaur aCCP and Abbott ARCHITECT anti-CCP assays was 96.84% (confidence interval 93.89–98.39%). The assay demonstrated no sample-tube type bias and no significant interference with the tested analytes or cross-reactivity with potentially cross-reacting conditions.

Conclusions: The results of these studies show good performance of the fully automated ADVIA Centaur aCCP assay and good agreement with the ARCHITECT anti-CCP assay.

A-375

Importance of Sealing the Samples during Incubation for an Aspergillus Galactomannan Antigen Assay

L. C. Reed, A. McShane, S. Wang. *Cleveland Clinic, Cleveland, OH*

Background: The galactomannan antigen is released in the circulation during active invasive aspergillosis. The Platelia Aspergillus Antigen assay is an FDA-cleared one-stage immunoenzymatic sandwich microplate assay which detects galactomannan in human serum or bronchoalveolar lavage (BAL) fluid (Bio-Rad Laboratories in Hercules, California). The test takes four major steps: 1) Serum or BAL fluid samples are heat-treated in the presence of EDTA to dissociate immune complexes and to precipitate proteins that could interfere with the test; 2) The treated samples and conjugate reagent are added to the microplate wells coated with monoclonal antibodies, and incubated at 37°C for 90 min. A monoclonal antibody-galactomannan-monoclonal antibody/peroxidase complex is formed in the presence of galactomannan antigen; 3) After wash to remove any unbound material, the chromogen solution is added, which reacts with the complex enzyme to form a blue color product; 4) After stopping the reaction with addition of an acid reagent, the absorbance (optical density or O.D.) was measured by a spectrophotometer set at 450 and 620/630 nm. When we first brought up the assay using an automated ELISA platform, we had to incubate the plate at step 2 without having the samples sealed. It was noted that the Cut-off controls were continuously outside the acceptable O.D. range of ≥ 0.300 and ≤ 0.800 as indicated in the package insert. We hypothesized that incubation without a plate sealer on the automated ELISA platform allowed the fluid in the plate wells to evaporate and caused elevated control results and failure to meet testing validity criteria. The aim of this work was to revalidate the assay with plate wells sealed during the 90 min incubation (step 2).

Method: Validation testing was performed using leftover patient BAL and serum samples in the laboratory and specimens obtained from a reference laboratory. Precision, method comparison, stability, and reference interval studies were performed and O.D. was closely monitored.

Results: Within-day and between-day reproducibility produced a 100% concordance with QC materials intended for negative and positive results. Qualitative method comparison resulted in a 97.5% concordance for serum specimens and 100% for BAL. Reference interval studies using culture Aspergillus free samples verified an index cutoff of <0.50 for negative results. The O.D. of each cut-off control was consistently ≥ 0.300 and ≤ 0.800 for all re-validation experiments.

Conclusion: A plate sealer was proven an important requirement during incubation at 37 °C for 90 min. This newly validated assay was validated for patient testing.

A-376

Development of a Novel Carrier Protein for Polyclonal Antibody Production

J. L. Miller[1], B. Bucher[1], R. Harney[2], G. Lundell[1], R. C. Bayer[3], I. Baburina[2], G. F. Krug[1]. *[1]Lampire Biological Laboratories, Pipersville, PA, [2]Saladax Biomedical Inc., Bethlehem, PA, [3]Lobster Unlimited LLC, Brewer, ME*

Background: Carrier proteins are critical in the antibody production process as they confer immunogenicity to poorly immunogenic compounds such as small molecules or peptides. Hemocyanins are copper containing proteins used for oxygen transport in arthropods and mollusks and are routinely utilized as carrier molecules due to their size, potent immunogenicity, and phylogenic distance from mammalian hosts/antigens. The most commonly used carrier protein is keyhole limpet hemocyanin (KLH). However, current immunization protocols often require alternating carrier molecules to prevent immunodominance of the carrier to the detriment of the antibody response to the hapten. Thus, there is a need for an alternative highly immunogenic carrier protein. Lobster hemocyanin (LH), is readily available as a byproduct from the food industry. Here, we evaluate the utility of lobster hemocyanin as a carrier protein of hapten antigens in comparison with KLH. **Methods**: Hemocyanin was purified from American lobster (*Homarus americanus*) serum. Peptide and protein antigens were covalently conjugated to LH and KLH using MBS. New Zealand white rabbits (N = 3 per immunogen) were immunized with either the KLH-antigen conjugate or the LH-antigen conjugate. Serum was collected at designated time points following immunizations and antigen specific antibody titers were measured by indirect ELISA. Antibody responses directed against the carrier molecule (LH or KLH), as well as cross-reactivity of anti-LH and anti-KLH antibodies, were also measured via indirect ELISA. **Results**: Pre-existing antibodies against LH were undetectable by ELISA in sera isolated from naïve rabbits (N = 9). Immunization with both LH and KLH conjugated antigens resulted in the production of antigen specific antibodies. There was no significant difference in the antibody titers generated against the peptide or protein antigens conjugated to LH or KLH. Antibody titers generated against LH were consistently less than that generated against KLH, however these differences were not significant at all time points. Anti-LH antibodies did not cross react with KLH, however sera from one animal immunized with KLH had low cross-reactivity to LH (titer <100). **Conclusions**: LH is an immunogenic and effective carrier protein that promotes the generation of rabbit polyclonal antibodies to conjugated haptens as effectively as KLH. Anti-LH antibodies do not cross-react with KLH, signifying that the LH epitopes are unique from those on KLH. Thus, LH is novel carrier protein that can be used as an alternative to or in conjunction with KLH.

A-377

CSF Free Light Chain Identification of Demyelinating Disease: Overcoming Challenges of Oligoclonal Banding

K. Gurtner, D. Murray, E. Shosha, D. Barnidge, S. Pittock, M. V. Willrich. *Mayo Clinic, Rochester, MN*

Background: Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS). Cerebrospinal fluid (CSF)-index and oligoclonal bands (OCBs) are common laboratory tests used in MS identification, employing isoelectric focusing (IEF) and nephelometry, each requiring paired CSF and serum. IEF is not only costly and labor-intensive, but also involves subjective interpretation and little consensus defining the amount of OCBs required for positivity. Our laboratory compared both free light chain (FLC) presence and various published calculations/indexes that suggest representation of humoral immune response, to determine a more sensitive, specific, and cost-efficient means of diagnosing MS.

Methods: 325 residual CSF and serum specimens were obtained after physician-ordered OCB testing (211 OCB negative, 114 OCB positive). Paired CSF and serum specimens were characterized using IEF on the SPIFE 3000 (Helena) platform, followed by band pattern interpretation requiring 4 or more CSF exclusive bands. FLC (Freelite® Human Kappa/Lambda, The Binding Site), albumin and total IgG (Siemens) were measured on paired specimens using a BNII nephelometer (Siemens). Calculations were performed based on combinations of analytes: CSF sum of Kappa and Lambda [(KFLC+LFLC)], kappa-index (K-index) [(CSF KFLC/serum KFLC)/(CSF albumin/serum albumin)], kappa intrathecal fraction (K-IF) {[(CSF KFLC/serum KFLC)-(0.9358 x CSF albumin/serum albumin)^0.6687)x serum KFLC)]/CSF KFLC)}, and CSF-index [(CSF IgG/CSF albumin)/(serum IgG/serum albumin)]. Positivity with each calculation/index was correlated to medical decision points after ROC curve analysis and chart-review. Precision, accuracy, measuring and reportable ranges, and carry-over studies for CSF KFLC were performed on an additional cohort of residual CSF specimens (n=360).

Results: Patients were categorized by clinical condition: Demyelination (n=67), Autoimmunity (n=53), Non-Inflammatory (n=50), Inflammation (n=38), Degeneration (n=28), Peripheral Neuropathy (n=24), Infection (n=13), Cancer (n=11), Neuromyelitis Optica (n=10), and Other (n=31). Each FLC measurement or calculation/index exhibited ≥90% agreement with OCB, with the exception of CSF LFLC and CSF-index, which only demonstrated 80 and 83% agreement, respectively. CSF KFLC measurement used alone exhibited 90% agreement with OCB, subsequently reducing the number of analytes measured and variables associated with calculations. When cases of demyelinating disease were reviewed, KFLC measurements showed 85% clinical sensitivity/69% specificity, whereas OCB exhibited 78% clinical sensitivity/76% specificity. BNII analytical imprecision studies produced an intra-assay precision of <10% (n=20) and an inter-assay precision of <20% (n=20), along with demonstrating the absence of carryover.

Conclusion: Results have shown that KFLC alone demonstrates comparable performance to OCB along with increased sensitivity for demyelinating diseases. Replacing OCB with KFLC for MS investigation would alleviate the need for serum IgG and albumin, CSF IgG and albumin, and calculated conversions. KFLC

measurement would allow the laboratory to decrease technologist bench time from 4 hours to 20 min/specimen, creating an automated set-up with reduced turnaround time, subsequently reducing the overall testing-related costs by 75%. CSF KFLC can overcome challenges associated with performance, interpretation, and cost of traditional CSF diagnostics, in turn, reducing costs to the patient while maintaining sensitivity and specificity supporting MS diagnosis.

A-378

Detection of antiphosphatidylethanolamine among cardiovascular patients with ELISA using synthetic antigens

Y. Zhang[1], S. Hou[2], X. Wang[1], G. Chen[1], Z. Zhou[1], M. Zhao[2]. *[1]National Center for Cardiovascular Diseases & Fuwai Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China, [2]Northwestern University, Chicago, IL*

Background: Antiphosphatidylethanolamine (aPE) is a form of "non-criteria"antiphospholipid syndrome (APS), where the presence of antiphospholipid antibodies has clinical symptoms consistent with APS but fall outside current standard tests. A major limiting factor in diagnosing aPE is a lack of standardized assays. This makes it a challenge for positively identifying patients and for investigating the prevalence, pathogenesis and impact of aPE. The goal of the current study was to determine the presence of aPE antibodies among cardiovascular patients with ELISA-based assays using synthetic phosphatidylethanolamine (PE) antigens.

Methods: A total of 400 continuous cardiovascular patients and 160 normal controls were enrolled. The presence of aPE antibodies, including cofactor-dependent IgG, IgM and IgA and cofactor-independent IgG and IgM, were examined using ELISA. The assays involved the use of synthetic PE species as antigens, including 18:1 PE for the detection of cofactor-dependent aPE isotypes and 20:4 PE as a major component for detecting cofactor-independent aPE isotypes. The lipid antigens were coated onto microtiter plates. The wells were rehydrated and blocked with BSA. Human plasma samples were added to the plates in duplicates at 1:100 dilution. Visualization was achieved using alkaline phosphate-conjugated anti-human immunoglobulin antibodies which were isotype-specific, and then a colorimetric reaction with p-nitrophenyl phosphate. The outcome of the aPE assays, based on a threshold of control mean plus 6 standard deviations, were used to calculate the percentage of aPE positive cases among cardiovascular patients, and the association with different clinical diagnoses and symptoms. **Validation:** The outcome of the aPE assays was compared with ELISA tests using conditions reported in the literature involving natural (egg yolk) PE as antigen. The remaining ELISA protocols were identical as described above. The percentages of aPE-positive cases and the coefficients of correlation between the two sets of aPE assays were determined. **Results:** Different PE species gave rise to distinct physical properties, which allowed the differentiation among sub-types of aPE antibodies with specificity. The use of synthetic PE species led to the identification of cofactor-dependent or cofactor-independent aPE cases, whereas when egg PE was used as antigen such differences could not be clearly resolved. Notwithstanding these differences, the overall ELISA results between the current and prior methods were significantly correlated, with Pearson correlation coefficients being 0.80, 0.79, 0.93, 0.82 and 0.93 for cofactor-independent IgG and IgM, and cofactor-dependent IgG, IgM and IgA, respectively. The data indicated that there were significantly higher incidences of aPE among cardiovascular patients (6.75%) compared to controls (1.25%). Cofactor-dependent aPE cases accounted for the majority of aPE cases among cardiovascular patients (6.0%) versus controls (0.625%). Overlap was identified between aPE and lupus erythematous, anticardiolipin and anti-beta2 glycoprotein I. However, the majority of aPE cases (88.37%) were not associated with lupus, anticardiolipin or anti-beta2 glycoprotein I, suggesting aPE may be an independent etiological entity.

Conclusion: The present data demonstrated the feasibility of using synthetic lipid antigens for aPE ELISA, which minimizes uncertainties associated with natural antigens. The aPE antibodies has a significant presence among cardiovascular patients.

A-379

Evaluation of Customizable Allergen Mixes on a New Immunoassay Technology Platform

E. Sinson, S. Ortega, S. Neath, R. Hockins, D. Canfield, T. Reid, E. Whitters. *Hycor Biomedical, Garden Grove, CA*

Background: Multi-allergen tests (or allergen "mixes") are readily available and sold by various diagnostic manufacturers. Often these mixes comprise a group of similar allergens such as trees, grasses, or foods that will be tested together. However, "one-size-fits-all" pre-manufactured mixes may not be best for initial evaluation of allergic disease given regional variations in allergenic sensitization. We are developing a novel *in vitro* IgE testing platform that enables a clinician to design a custom mix of 3-6 allergens for each patient, providing flexibility across a menu of allergens.

Methods: The new system is a fully-automated chemiluminescent immunoassay platform to quantify specific IgE (sIgE) concentrations in human serum. The system utilizes magnetic microparticles to which allergens are coupled by a process called 'on-board kitting'. The assay then adds 4uL of serum to the coated beads to quantify sIgE concentrations for that allergen. By the same on-board kitting process, multiple allergens can be coupled to these beads to create a mix test. This study was designed to determine sensitivity and specificity of samples across individual allergens and multi-allergen mixes. Using 36 known low-reactivity samples (0.3 - 0.5 kU/L), sensitivity was calculated on a 6-allergen mix compared to the results of the same 6 individual allergens. Additionally, mono-sensitized samples were evaluated for 3-, 4-, 5-, and 6- allergen mixes to determine whether sensitivity is affected for larger mixes. 30 negative samples were evaluated across 6 allergens on both individual and multi-allergen tests.

Results: Sensitivity for a 6-allergen mix was calculated at 97.2% with 35/36 samples recovering positive. One sample reported a 0.37 kU/L sIgE concentration for Bermuda Grass when tested individually but negative in the mix test. Specificity for a 6-allergen mix was calculated to be 96.7% with 29/30 samples negative. Across 3-, 4-, 5-, and 6- allergen custom mixes, 9 mono-sensitized positive samples and 5 negative samples were tested. Positive sample recovery was consistent across each mix; however, in the 3-allergen mix, 1 sample tested negative. This sample reported 0.44 kU/L sIgE concentration for House Dust Mite when tested individually but was negative in the 3-allergen mix test. Other mono-sensitized samples with lower kU/L values did successfully report positive, indicating that the multi-allergen test can recover low sIgE concentrations. Sensitivity for 3-, 4-, 5-, and 6- allergens mixes was 89%, 100%, 100%, and 100%, respectively. Specificity for 5 negatives across each allergen mix number was 100%.

Conclusion: The ability for a clinician to select the most appropriate allergens that are geographically relevant and/or optimized based on the patients' medical history and physical work-up is a step toward personalized diagnostics. The study findings demonstrate that multi-allergen mixes up to 6-allergens have good sensitivity for a selection of low-reactive samples and across different mixes.

A-380

Characterization of B- and T-cell Immune Repertoires Using Anchored Multiplex PCR and Next-Generation Sequencing

J. Eberlein[1], T. Harrison[1], J. Simms[2], M. Wemmer[1], L. Griffin[1], B. P. Culver[1], L. Johnson[1], B. A. Kudlow[1]. *[1]ArcherDX, Boulder, CO, [2]Q2 Solutions, EA Genomics, Morrisville, NC*

Introduction: NGS-based analysis of the immune repertoire (IR) is a powerful tool to monitor disease, adaptive immune responses to disease, vaccination and therapeutic interventions. IR characterization by NGS usually requires large primer panels to cover its extensive combinatorial diversity, and a complex system of synthetic controls to account for differential amplification efficiency across segment combinations. Anchored Multiplex PCR (AMP™) uses molecular barcoded (MBC) adapters and gene-specific primers (GSPs), enabling NGS-based immune chain mRNA interrogation from a single side. This eliminates the need for opposing primers that bind within the highly variable V-segment, eliminating clone dropout due to somatic hypermutation. Here, we describe AMP-based NGS assays for IR characterization, Immunoverse™ IGH and TCR, which utilize a minimal set of unidirectional GSPs and MBC adapters that reduce amplification bias.

Methods: The quantitative reproducibility and sensitivity of our assays was validated using mRNA isolated from PBMCs of healthy donors, B-cell chronic lymphocytic leukemia donors and formalin-fixed paraffin-embedded (FFPE) tissue.

Results: Both assays demonstrated high reproducibility between replicates with quantitative clone tracking down to 0.01%. The ability to determine isotype, clonotype and IGHV mutational status in a single assay was demonstrated. Preliminary TCR assay data indicates that CDR3 sequence capture is possible from FFPE tissue with clonotype calling being driven by input quantity, T-cell content, and, to a lesser degree, mRNA quality.

Conclusions: AMP-based NGS with MBC quantification and error-correction is a powerful method to characterize the immune repertoire.

A-381

TSG-6 Neutralizing Monoclonal Antibodies (MAb) As A Potential Therapeutics For Asthma Treatment

N. Albtoush[1], A. Abbadi[1], S. Merkoulov[1], M. Lauer[1], T. Glant[2], K. Mikecz[2], V. Hascall[1]. *[1]Cleveland Clinic, Cleveland, OH, [2]Rush University Medical Center, Chicago, IL*

Background: Asthma, a long-term respiratory disease, is characterized by increased hyaluronan (HA) extracellular matrix (ECM) and infiltration of inflammatory cells. The HA ECM has been modified by formation of covalently bound heavy chain proteins (HCs) to HA. This modification is mediated by a unique enzyme called tumor necrosis factor-stimulated gene 6 (TSG-6) that is upregulated in inflammations. TSG-6 transfers HCs from inter-α-inhibitor (IαI), a serum proteoglycan with 2 HCs on the bikunin chondroitin chain, onto HA forming the HC-HA matrix. The resulted HC-HA matrix extensively recruits Th2 lymphocytes, eosinophils and macrophages that accumulate in the airway mucosa and submucosa, which increases asthmatic symptoms and disease progression. In contrast, a milder form of asthma with significantly less inflammation and less airway hyperactivity has been shown in our TSG-6 knockout mouse model that lacks the ability to form HC-HA matrix. Accordingly, we propose to lower the levels of HC-HA crosslinking by inhibiting the action of TSG-6 through a novel immunotherapy approach to manage and suppress the Th2 cell-mediated inflammatory response in asthmatic airways. **Methods:** We have tested a panel of murine TSG-6 monoclonal antibodies (mAb) referred to as NGs (NG1-NG7) in a TSG-6/HC transfer assay. Recombinant TSG-6 is mixed with HC donor IαI to form the TSG-6-HC intermediate. In the presence of HA14, a short HA oligosaccharide, TSG-6-HC irreversibly transfers the HC to HA14 to form HC-HA14 with MW of about 75 kDa. The absence of the HC-HA14 and the continued presence of IαI will indicate that TSG-6 HC transfer was inhibited by NG mAb. Additionally, time course studies were performed to determine reaction kinetics from 0 to 6 hours in the presence and absence of TSG-6:NG4 and TSG-6:NG5 at equimolar ratios. All samples were probed with the Dako IαI Ab and analyzed by Western blot. Moreover, the binding affinities of NGs to TSG-6 were measured by surface plasmon resonance (SPR) technique. **Results:** Our initial results show, a 75 kDa band for NG1, NG2, NG3, NG5, NG6 and NG7 indicating that they did not prevent HC transfer to HA14. In contrast, the band is not present for NG4 indicating that NG4 binding to TSG-6 is a very effective inhibitor for HC transfer. In a time course, the NG4 mAb showed nearly complete inhibition of HC-HA14 within the first 4 hours. The SPR results revealed a tight and almost irreversible binding of TSG-6 to NG4 with (Kd ~ 50 nM). NG5 bound to TSG-6 weakly (Kd ~300 nM) and was reversible. **Conclusion:** We identified NG4 as the most active neutralizing mAb that inhibits HC transfer to HA by TSG-6, which will be used in subsequent experiments to determine if it can prevent or greatly inhibit formation of a HC-HA matrix in asthmatic airway smooth muscle cell culture models.

A-382

Comparison of fecal lactoferrin and calprotectin as screening markers of inflammatory bowel disease

K. W. Cradic, M. Willrich, M. R. Snyder. *Mayo Clinic, Rochester, MN*

Background: Inflammatory response in the intestines results in activation of neutrophils, leading to release of the cytoplasmic granular proteins calprotectin and lactoferrin. Screening assays for these biomarkers in feces can be used to distinguish between inflammatory and non-inflammatory conditions. Previous reports suggest that lactoferrin and calprotectin perform equally as markers of intestinal inflammation. However, these studies used differentiated populations of patients with and without inflammatory diseases. These populations do not reflect the majority of patients who present with GI symptoms, making it necessary to evaluate the utility of lactoferrin and calprotectin as screening tests in a cohort of patients for whom disease status is uncertain. Therefore, the aim of this study was to determine the concordance of calprotectin and lactoferrin by various assays in a routine clinical setting.

Methods: Residual stool samples (n=33) were collected from specimens submitted for routine calprotectin testing. In addition to the clinical lab's calprotectin assay (Inova Diagnostics; San Diego, CA), specimens were tested using a second calprotectin ELISA and a point-of-care kit (both from Immundiagnostik; Bensheim, Germany). Each sample was also tested for lactoferrin using a commercially-available ELISA (ALPCO; Salem, NH). All testing was performed according to manufacturer's instructions. Results were compared qualitatively using 50 mcg/g as the cut-off for all calprotectin assays and 7 mcg/g as the cut-off for lactoferrin. Results between calprotectin assays were also compared quantitatively by linear regression.

Results: Qualitatively, the calprotectin ELISA and point-of-care device, from the same manufacturer, showed the highest agreement at 97%. The current clinical calprotectin assay demonstrated concordance with the second ELISA and the point-of-care device at 88% and 84%, respectively. However, quantitative analysis showed that the clinical assay correlated best with the second ELISA (slope = 3.3 [95% CI 2.6 - 5.9], intercept = -18.7 [95% CI -56.7 - 1.0], r = 0.761) and least with the point-of-care device (slope = 3.99 [95% CI 2.7 - 6.6], intercept = -29.9 [95% CI -61.3 - -8.5], r = 0.390). Correlation between the second ELISA and the point-of-care device was also calculated (slope = 1.07 [95% CI 0.5 - 1.7], intercept = -1.4 [95% CI -21.2 - 75.4], r = 0.604). Lastly, qualitative concordance of lactoferrin with the clinical calprotectin assay was 82%. Similar agreement with the second calprotectin ELISA and the point-of-care device, at 76% and 73% respectively, was observed. When compared to calprotectin, of the 6 discrepant results, 5 (83%) were negative for lactoferrin, despite being abnormal by all 3 calprotectin assays.

Conclusion: Various calprotectin assays, including a point-of-care device, showed qualitative concordances >80%. However, quantitative agreement was poor, suggesting that the assays do not have standardized calibrations and cannot be used interchangeably. Lactoferrin showed lower concordance with calprotectin, regardless of methodology. The majority of discordant samples were abnormal by calprotectin and negative for lactoferrin, suggesting that calprotectin is a more sensitive marker for identifying patients with a higher likelihood of active intestinal inflammation. For these reasons, calprotectin should be considered first for screening patients suspected of having IBD.

A-383

Evaluation of the impact of choosing wisely campaign recommendations on ANA and subserology testing in current practice

A. Muthukumar, A. Alambo, B. Crook. *UT Southwestern Medical Center, Dallas, TX*

Background: American college of Rheumatology recommended the following guidelines for ANA and subserology testing as a part of choosing wisely campaign: A stepwise approach to ANAS testing in which ANAS to be tested only if ANA is positive and there is clinical suspicion of immune disease. The exception to this rule when there is suspicion of myositis in which case anti-Jo-1 can be tested or occasionally, anti-SSA can be tested in the setting of lupus or Sjogren's syndrome. Since, ANA is a screening test and not for monitoring, serial measurement of ANA is not recommended, especially in those with an existing diagnosis of immune disease. Given this background, the purpose of the study was to review how frequently ANA and ANAS testing were ordered together and how many were repeats, especially those orders from rheumatologists. **Methods:** All patients from our institution who underwent ANA and ANAS testing from January 2011 to February, 2017 were evaluated through retrospective chart review. ANA and dsDNA testing were performed by indirect immunofluorescence assay. ENA panel testing (Smith, SSA, SSB, RNP, Scl-70, and Jo-1) and anti-centromere testing were performed by multiplex flow immunoassay. ANA and ANAS tests were available for routine ordering by all our physicians.

Results: A total of 25319 patients had about 26992 test orders for ANA during the study period. The mean age of the study population was 54 and the women to men ratio was 2:1. The majority of the test requests came from specialties like Neurology and Rheumatology. Year wise orders for ANA test remained almost the same throughout the study period (4393). A total of 2910 ANA test orders (11%) were repeats, with 2170 being repeated twice and 611 orders being tested thrice. 6 patients were tested more than 10 times during the study period. Total requests for dsDNA and ENA testing during the 6 year study period were 12323 and 9964 respectively. About 25% of test orders had either dsDNA or ENA ordered simultaneously with ANA and about 14% of test requests had all 3 tests ordered together. Nearly 3328 orders for ENA were placed in patients who had previously negative result for ANA (12%). Likewise, dsDNA orders were placed in 3055 encounters when ANA was negative (11%). Rheumatologists placed as many orders as non-specialists for dsDNA (15%) and ENA testing (13%) in patients with previous negative ANA results. The percentage requests from rheumatogists for simultaneous testing of ANA and ENA, ANA and dsDNA as well as all 3 tests together was more than the twice the requests from non-specialists. The number of repeat orders for ANA were significantly lower (<1%) for the rheumatologists group. **Conclusion:** ANA repeat orders as well as simultaneous orders for ANA and ANAS testing remain significantly high in our hospital. The percentage of simultaneous orders for ANA and ANAS testing was even substantially higher in the rheumatologists group. Further education and placing restrictions on ordering of these tests in the electronic medical record system may be the most effective way to reduce unnecessary test orders.