**Immunology**

**A-332**

Paroxysmal, but not persistent, atrial fibrillation is associated with increased levels of transforming growth factor beta 1

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**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 calibrator 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±67.7 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 mg/mL vs. 25.2±3.2 mg/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 anti-fibrotic 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

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**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:** 171 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 R²=0.973 for MPO and R²=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>
<thead>
<tr>
<th>Concordance (95% CI)</th>
<th>INOVA (site 1)</th>
<th>BioPlex (site 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO</td>
<td>97.2% (85.9 to 99.5%)</td>
<td>76.5% (66.2 to 84.4%)</td>
</tr>
<tr>
<td>PR3</td>
<td>94.4% (83.9 to 98.5%)</td>
<td>76.5% (66.2 to 84.4%)</td>
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**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/cid 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 1 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+ 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 tolereize 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

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**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 perform 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 PLAR2 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.

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**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 an ELISA was used as initial testing, 39% of 216 responding laboratories reported as positive/negative in 67%, in units by 21%, optical density by 3%, and using HEp-2 cell lysate as substrate, 4% a lysate of the HEp-2000 cell line (which is an ELISA was used as initial testing, 39% of 216 responding laboratories reported as positive/negative in 67%, in units by 21%, optical density by 3%, and

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

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**A-340 Abbott Alinity i Sigma Metrics and Precision Profiles for Immunoassays**

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**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 laboratorys 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: sigama = (%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 the ARCHITECT i2000 systems. The mean concentration of the Alinity i-series results were regressed versus the mean ARCHITECT i2000 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

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**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 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 of 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.
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 immunoassay results 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**


**Background:** Bence Jones proteins or monoclonal immunoglobulin free light chains (FLCs) hold an important supportive role in diagnosis, prognostication, 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 immunoenrichment-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 nanobodies 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 MicroflexTM) in automated acquisition mode. Mass spectra were interrogated for M-proteins and isotopes.

**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=2.5-34) 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 rFLC 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-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.
Tuesday, August 1, 9:30 am – 5:00 pm

Immunology

A-344

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


Background: Krebs von den Lungen-6 (KL-6), which is a mucin-like glycoprotein 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 bronchiectasis 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-relatedILDs (CTD-ILD) than other groups. KL-6 levels were negatively correlated with PFTs [FVC, DLco, TLC and 6MWWT]. In IPF patients, there were statistically significant correlations with all PFT results, but in nonspecific 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

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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 Dianogistics 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

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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 polyclonal 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 other 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 ACRA (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


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.

A-350


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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 4000 U/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 98.9%, 93.3%, and 92.2% accuracies to the three ELISA kits. The sensitivity, specificity, and accuracy of the Denka kit compared with the clinical diagnosis were 91.1% (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

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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 Freekit 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. Denning 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.
Analysis of Serum Immunoglobulins by Capillary Electrophoresis

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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, and total λ Ig were calculated with graphical algorithms. For most ELPS, the β-γ boundary was defined as the valley between the β- and γ-Ig. Regression yields an equation relating the feature to total IgA, IgG, and IgM. Seven features - curve heights or areas - of the ELP were chosen for regression analysis. Each curve feature was regressed against IgA, IgG, and IgM. Regression coefficients for β-Ig and λ-Ig should be complements of each other. The placement of the β-γ boundary plays a critical role in the regression of β- and γ-Ig. 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 0.95. The γ-peak height is directly proportional to IgG with a correlation of 0.95, but the γ-peak height is not significant. Most sets of simplex equations included the β-γ boundary height. 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-γ peak. The smallest R-square for any of the regressions was used as the β-γ boundary. Three sets of simplex equations produced correlations better than 0.97 for the IgG and IgM of ELPs, but none produced an IgM correlation better than 0.50. DISCUSSION: The sum of β- and λ-Ig should equal the sum of G, A, M-lgs for further analysis. Simplex 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 further analysis. Each curve feature was regressed against total IgA, IgG, and IgM. Regression yields an equation relating the feature to each Ig. The β-γ boundary was defined as the valley between the β- and γ-Igs. Regression coefficients for β-Ig and λ-Ig should be complements of each other. The placement of the β-γ boundary plays a critical role in the regression of β- and γ-Igs. 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 IgM of ELPs, but none produced an IgM correlation better than 0.50. DISCUSSION: The sum of β- and λ-Igs should equal the sum of G, A, M-lgs, thus regression coefficients for β- and λ-Igs 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-Igs and the height of the boundary. Large elevations of γ-Ig shift the valley between the β-γ zones upward and toward the anode. A 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**

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**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 EP07-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-EP07-A2, the assay was tested with potential cross-reactants such as human serum amylloid 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**

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**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**

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**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 EP09-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±0.64%. 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 System 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 Immuno blot and Application on BioCLIA® 4G Allergy Assay**

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**Background:** In vitro allergen-specific IgE assays, integrated with component-resolved diagnostics (CRD), offer allergists an allergen-sgE sensitization profile with insight into clinical relevance such as risk 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 to supplement 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 quantitatively profile 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 extract sources were screened for relative component content by quantitative SDS-PAGE. Allergenic components, including Pru p1, 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 p1 and the patient panel reactivity was re-assessed. Results: A panel of eighteen F95-positive samples was identified. The sensitivity of this patient panel varied across different F95 raw materials, ranging from 89% for F95 1, 42% for F95 2and 53% for F95 3. WES positive signals were identified from unidentified component of ~95 kDa in F95 1 material, an unidentified component of ~66 kDa in F95 2 material, and both ~66 kDa and ~95 kDa in F95 3 material. All materials were essentially missing Pru p1. Eleven samples (61%) showed reactivity to Pru p1 (sIgE ≥ 0.35 IU/ml), with a response profile different from those of the three F95 materials. Adding defined ratios of Pru p1 increased the 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-PAGE and capillary Western blot, raw material component ratios can be identified in a patient-specific manner. Through guided adjustment of allergenic components in 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 Sensitive Method for the Determination of Anti-CCP on the HOB 4G BioCLIA® 1200 Automated Immunoassay Analyzer

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Background: Rheumatoid Arthritis (RA) is a common, systemic autoimmune disease affecting between 0.5-1 % of the adult population. Patients with RA has been found to develop endemic auto-antibodies to cyclic citrullinated peptides (anti-CCP), which are used as a marker in the diagnosis of early RA. Since the first report in 1999 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 Xiche 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 (CILD) (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 2-4000 RU/mL, and the LOD was 0.064 RU/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% (110/110). The total agreement of BioCLIA® & ELISA was 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% (11/120), 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® 200 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.

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

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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 (NVT). The toluidine red unheated serum test (TRUST), T. pallidum particle agglutination assay (TPPA) plus chemiluminescent immunoassay (CLIA) are employed in our hospital. There have not been 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), slight (0-0.2), or poor (<0).

Results: Overall, 1477 subjects had TRUST+/TPPA+/CLIA+ results, and 527 subjects had TRUST-/TPPA-/CLIA- results. 6 subjects were TRUST+/TPPA-. Among these subjects only 1 was CLIA+, which was discordant with the TPPA test result. These 5 TRUST+/TPPA-/CLIA- 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-/TPPA+/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 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
**A-362**

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

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**Background:** Anti-mitochondrial IgG antibodies are one of the primary auto-antibodies present in patients with Primary Biliary Cirrhosis (PBC) and in intra-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 (BICOA-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 immunofluorescence assay (IFA) results, were analyzed by both BioCLIA® and ELISA (from a different international manufacturer). 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 study, the CV% was 4.38% for intra-assay and 5.05% for inter-assay, respectively. Bilirubin (to up 50 mg/dL), hemoglobin (to 400 mg/dL), bilirubin (to 400 mg/dL), and lipid (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% (299/300) & 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 offers 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**

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**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 cardiocardiolipin IgG, IgA, IgM and M9. The BioCLIA® aCL kits were more accurate, sensitive, quantitative and automated than traditional ELISA or LIA kits. **Methods:** The analytical performances including 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 using 300 clinical 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 patients sample, 102 systemic lupus erythematosus (SLE) patient samples, 150 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 GAD and IA-2 antigen were 0.41 IU/mL, 0.13 IU/mL, 0.06 IU/mL, and 0.08 IU/mL, respectively. In the presence of GAD samples, the sensitivity of GAD and IA-2 antibodies was 96.7% (58/60) and 98% (49/50), respectively. In the presence of IA-2 antigen, the sensitivity was improved to 99.0% (59/60) and 98% (49/50), respectively. 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 TIDM autoantibodies and would be a well-accepted choice in the diagnosis of TIDM.

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Performance Evaluation of Der p 1 and Der p 2 specific IgE in Chinese House Dust Mite Allergy Patients on the HOB BioCLIA® Analyzer

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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 Dermatophagoides farinae 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 2 Assays 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 near-range ranging from 0.01-1000 IU/mL. The LoD of BioCLIA® sIgE - Der p 1 and Der p 2 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 p 1 and Der p 2 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 100 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 2 performed against D1 samples were 69.8% and 72.3 %, respectively. HOB BioCLIA® Der p 1- and Der p 2-sIgE Assays also agree well with Phadia ImmunoCAP Der p 1- and Der p 2-sIgE Assays, with positive agreements 95.2% and 97.2%, total agreement 96.8% and 98.4%, respectively. Regression analysis between HOB BioCLIA® and ImmunoCAP assays, the Der p 1 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® system offers 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 elicitation 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.
Tuesday, August 1, 9:30 am – 5:00 pm

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

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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 (EQ5551-9601-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 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%.

Conclusion: The EUROIMMUN Neurogranin ELISA kit was successfully validated as per the CLSI guidelines. The assay kit is suitable for performing testing on CSF samples to assess the concentration of Neurogranin.
A-372
Assessing the Laboratory Protocol for the Hevylite® Assay on a Multiple Myeloma Patient Cohort

Background: The Hevylite® assay (HLC) quantifies intact immunoglobulin (Ig) heavy and light chain pairs and is complimentary 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 difficultly 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=59), 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 (HLLC) 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, HLC 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 ratios and HLLC.

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 HLLC concentrations. The median recovery was 82% for IgGκ (range: 35-136%), 80% for IgGλ (50-140%), 135% 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, 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, and 110% for IgAλ (41-164%). A total of 254 measurements (49%) were outside the 80-120% recovery range. 170 measurements were assessed for antigen excess misses, with only one, the IgAκ measurement, and 110% for IgAλ (41-164%). A total of 254 measurements (49%) were outside the 80-120% recovery range.

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%.
Results: The ADVIA Centaur aCCP assay measured anti-CCP IgG concentrations from 0.40 to 200.0 U/mL, with LoD determined to be 0.40 U/mL. The assay was determined to be linear from 0.40 to 200.0 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
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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, 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 the 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

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 phylogenetic 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 immunomodulation 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 naive 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 haptenics 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 KLH in conjunction with KLH.

A-377
CSF Free Light Chain Identification of Demyelinating Disease: Overcoming Challenges of Oligoclonal Banding

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 nephelometric (NF) requiring paired serum and CSF. 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), kappalambda intrathelial fraction (K-LF) ([CSF KFLC/serum KFLC]/0.9538×CSF albumin/serum albumin)/0.6857×serum albumin], 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), Autoimmune (n=41), Inflammatory (n=55), Inflammatory-Non-Inflammatory (n=5), Neurodegeneration (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 FLLC 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/99 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

Immunology
Tuesday, August 1, 9:30 am – 5:00 pm

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Antiphosphatidylethanolamine among cardiovascular patients with ELISA using synthetic antigens

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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-dependent 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 erythematosus, antiphospholipin 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 have a significant presence among cardiovascular patients.
**A-381**

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

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**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-a-inhibitor (Ii), a serum proteoglycan with 2 HCs on the bimukin 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 Ii 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 Ii 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 IiAb 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**


**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 or greatly inhibit formation of a HC-HA matrix in asthmatic airway smooth muscle cell cultures.

**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 two second ELISA and the point-of-care device was also calculated (slope = 1.07 [95% CI 0.5 - 1.7], intercept ~ -1.14 [95% CI -21.2 - 17.5], 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.