Algorithm to avoid delay in the diagnosis of multiple myeloma in patients with incidental clinical findings at emergency service

J. L. Garcia de Veas Silva, M. Lopez Velez, T. De Haro Romero, A. Espuch Oliver, J. Garcia Lario, T. De Haro Muñoz, Hospital Universitario Campus de la Salud, Granada, Spain

Background: The presence of incidental clinical findings (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia, acute kidney injury) related to Multiple Myeloma (MM) in Emergency Service and Primary Care should be studied for screening the existence of a possible MM. A quick panel based on serum protein electrophoresis (SPE) and quantification of serum free light chains (sFLC) enables sensitive quantification of monoclonal component in the study of MM. The application of this screening panel in patients with these incidental clinical finding without other diagnosis can help us to efficiently detect a possible MM in much sooner times.

Methods: we studied three patients admitted to Emergency Service where we found incidental clinical finding characteristic of multiple myeloma (anemia, hyperproteinemia, intense bone pain). Sera of the three patients were sent to the Laboratory of Immunology for the screening of a monoclonal gammopathy. SPE were performed on CAPILLARYS 2 (Sebia) and the sFLC were measured by FREELITE (The Binding Site) turbidimetric assay.

Results:
Case 1 (Man, 68 years) Clinical finding: macrocytic anemia (9.0 g/dl hemoglobin), rouleaux formation of erythrocytes, discrete pancytopenia. Protocol SPE+sFLC: strong peak in SPE (0.10 g/dl), sFLC ratio very altered (free kappa=14450 mg/l, free lambda=4.9 mg/l, ratio=2949) and immunoparesis. Diagnosis: Light Chain Kappa Multiple Myeloma Stage 3 ISS
Case 2 (Woman, 65 years) Clinical finding: hyperproteinaemia (12 g/dl), hyperviscosity and thrombocytopenia. Protocol SPE+sFLC: large peak (3.28 g/dl), altered sFLC ratio (free kappa=617 mg/l, free lambda=11.1 mg/l, ratio=55.59) Diagnosis: Multiple Myeloma IgG Kappa Stage 2 ISS
Case 3 (Woman, 64 years) Clinical finding: intense back pain Protocol SPE+sFLC: large peak (3.22 g/dl), altered sFLC ratio (free kappa=3.15 mg/l, free lambda=102 mg/l, ratio=0.031)
Diagnosis: Multiple Myeloma IgA Lambda Stage 3 ISS

Conclusions: In the context of clinical symptoms (bone pain, pathologic fractures, anemia, hyperproteinemia, hypercalcemia) that are alerts to suspect multiple myeloma it is advisable to apply the protocol (SPE+sFLC) for the screening of monoclonal gammopathies in patients without obvious clinical diagnosis. The combination of SPE and sFLC yields a fast and highly sensitivity approach in the screening of monoclonal gammopathies.
**Paediatric reference intervals for total IgG and IgG subclass concentrations on the OptiLite® automated turbidimetric analyser**

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**Background:** Total serum IgG consists of four structurally similar IgG subclasses (IgGc); IgG1-4. Concentrations of IgGc’s vary with age, most noticeably in paediatric populations as the immune system matures. The measurement of IgG and IgGc concentrations can aid in the identification of certain immune system disorders and immunodeficiencies. Determination of age-specific normal reference intervals for IgG and IgGc antibodies is therefore essential to aid assessment of normal and abnormal concentrations. In the present study we report paediatric reference intervals for IgG and IgGc concentrations on the OptiLite® turbidimetric analyser.

**Methods:** Healthy controls were selected at Hospital Universitari Vall d’Hebron, Barcelona, Catalonia, Spain (n=145, median age 8 years, range 0-18). Patients with immune-mediated disorders or clinical syndromes were excluded. The study was approved by the Ethics Committee of Hospital Universitari Vall d’Hebron, Barcelona, Spain (PR_14/134.2011). IgG and IgGc concentrations were measured on an OptiLite turbidimetric analyser (The Binding Site Group Ltd, Birmingham, UK).

**Results:** Median total IgG and IgGc concentrations for age groups 0-2, 3-4, 5-9, 10-14 and 15-18 years are provided in Table 1. The trend of concentrations across all age groups was IgG1>IgG2>IgG3>IgG4. Significant increases in concentration were observed for total IgG, IgG2 and IgG4 between the 0-2, 5-10 and 14-18 years age groups (p<0.05). There was a strong correlation between the total IgG concentration and summation of the IgGc concentrations (R²=0.89, p<0.0001, y=0.98x+14.51).

**Conclusion:** We have generated age-specific reference intervals in healthy children for total IgG and IgGc measurements using the OptiLite turbidimetric analyser. These intervals will help identify individuals with abnormal concentrations and thus will aid in the diagnosis of both primary and secondary immunological disorders.

<table>
<thead>
<tr>
<th>Median IgG and IgGc concentrations in a healthy paediatric population. Concentrations in mg/dL.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>0-2</td>
</tr>
<tr>
<td>3-4</td>
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<td>5-9</td>
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<td>10-14</td>
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<td>15-18</td>
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</table>

**Distribution of PTPN22 1858CT (R620W) polymorphism in Mexican older adults with frailty syndrome and its relationship with clinical parameters**

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**Background:** Frailty is a late-life syndrome of etiology unknown, characterized by muscle weakness, weight loss and fatigue. Even though the knowledge of the pathophysiological mechanisms underlying frailty remains limited, evidence suggests that inflammation has a major role in the pathophysiology of frailty. Recent studies have shown alterations in the activation and differentiation of CD4 T cells in older adults, changes made mainly in the different events of the T-cell receptor signaling cascade. The tyrosine phosphatase protein non-receptor 22 (PTPN22) gene encodes the protein tyrosine phosphatase lymphoid (LYP), an important molecule in the immune system and actively involved in the negative regulation of the T cell activation. So, based in these findings, we hypothesize that genetic variants within PTPN22 gene could affected the normal function of LYP. **Objective:** To analyze the association of PTPN22 1858CT (R620W) polymorphism with the frailty syndrome in Mexican older adults and its relationship with clinical parameters.

**Methods:** One thousand twenty-four elders were interviewed, 743 accepted blood sampling, 114 were excluded by incomplete data for frailty. Frailty was defined as the presence of ≥3 of five components (weakness, slowness, lack of energy, weight loss, fatigue). Genomic DNA was extracted from the peripheral blood of all subjects (n=629). PTPN22 1858CT (R620W) polymorphism was genotyped by Real Time PCR with pre-designed TaqMan Probes in the StepOne Plus kit. Statistical analysis was carried out using SPSS v20.0. **Results:** The average age 78 ± 6.0 years and 52.5% were women. Frailty subjects showed low mental state (p<0.001), greater disability for activities of daily living and instrumental (p<0.001) and low economic perception (p<0.001). Genotypic and allelic frequencies for the PTPN22 1858CT (R620W) polymorphism did not show significant differences between study groups (p=0.05). Moreover, we evaluated the clinical characteristics according to each genotype in each group and we observed that the pre-frail subjects carrying the CT genotype had a higher percent of weight loss than the carriers of the CC genotype (p=0.028), whereas in the frail subjects, carriers of the CC genotype had a higher percent of low physical activity level than those with the CT genotype (p=0.013). **Conclusions:** Our results suggest that the PTPN22 1858CT (R620W) polymorphism is not relevant in the genetic susceptibility for frailty syndrome in Mexican elderly. However, it was found that there is a significant and independent relationship between the polymorphism and weight loss and low physical activity level, two of the clinical components of the fragility phenotype.

**Abbott Alinity i System Sigma Metrics for Immunoassays**

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**Background:** Assay performance is dependent on the accuracy and precision of a given method. These two attributes can be combined into a sigma metric, providing a simple value for laboratorians to use in evaluating test methods. In addition to sigma metrics, precision profile charts can be used to visually assess the precision performance of a product across a concentration range. Sigma metrics were determined for more than 12 immunoassays tested on the Alinity i system. In 2017, a separate and distinct subset of 13 immunoassays were analyzed and presented as an AACC poster using similar methods. **Methods:** A sigma metric was estimated for each assay and was plotted on a method decision chart. The sigma metric was calculated using the equation: sigma = (%TEa - |%bias|) / %CV . A precision study was conducted at Abbott on each assay using the Alinity i system 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, 100-200 sample measurements with concentrations spanning the assay’s measuring interval were tested in duplicate at Abbott on the Alinity i and ARCHITECT i2000SR systems. The 1st replicate from the Alinity i system was regressed versus the mean ARCHITECT i2000SR concentration and a Passing-Bablok or weighted Deming regression model was fit. Using the regression model, the %bias was estimated near a critical concentration level. 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 system and the ARCHITECT i system, where the ARCHITECT i system overlaid with the Alinity 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 system immunoassays had sigma metrics greater than 5. The precision performance on the Alinity i system was considered acceptable 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.
Tuesday, July 31, 9:30 am – 5:00 pm

Immunology

A-335

Effects of resolvin D1 and E1 on systemic and hepatic inflammation caused by ethanol and LPS challenge: a time course analysis


Background/Purpose: Alcoholic liver disease (ALD) is a major health problem in the US and worldwide. ALD encompasses a variety of liver pathologies, including steatosis, an early stage of the disease, and steatohepatitis, a more progressive stage with both hepatic and systemic inflammation. Currently, there is no FDA-approved therapy for any stage of ALD. Resolvin D1 and E1 are lipid mediators derived from n-3 polyunsaturated fatty acids, and exhibit anti-inflammatory and pro-resolution effects in several pathological conditions. Herein, we tested the hypothesis that resolvins will attenuate liver injury caused by ethanol (EtOH) and lipopolysaccharide (LPS) administration via reduction of systemic and hepatic inflammation in an animal model of ALD.

Methods: C57BL/6J male mice received EtOH by oral gavage (EtOH-treated mice) or an isocaloric/isovolumetric maltose-dextrin solution (control mice) for 5 consecutive days. LPS was administered 24h following the final EtOH gavage along with either resolvin E1 (RvE1) or D1 (RvD1). Animals were euthanized 4h or 24h later. Liver injury was evaluated by plasma ALT activity. Hepatic neutrophil infiltration was evaluated by chloroacetate-esterase staining. Plasma cytokines/chemokines were measured by the multiplex magnetic bead-based Luminex immunassay. Hepatocellular injury at 24h, suggesting a potential pro-resolution effect, and thus may be a promising novel therapy for ALD.

Results: Compared to plasma ALT levels in control mice (16 ± 0.8 U/L), EtOH and LPS administration significantly increased ALT levels at 4h (74.71 ± 6.03 U/L), and to a greater magnitude at 24h post-LPS (305 ± 64 U/L). EtOH+LPS treatment synergistically increased plasma ALT as compared to EtOH alone (~12-fold) or LPS alone (~5-fold). Importantly, the EtOH+LPS-induced plasma ALT at 24h was significantly attenuated by RvD1 (141 ± 49 U/L) and to a lesser extent by RvE1 (209 ± 49.3 U/L). A Luminex immunassay revealed that the EtOH+LPS-mediated induction of plasma pro-inflammatory mediators, including TNF-α, IL-6, ILX, CXCL-1, MCP-1 and MIP-2, peaked at 4h, but remained elevated at 24h as compared to control animals. Notably, TNF-α was significantly decreased by both RvE1 and RvD1 at 24h, and a similar trend was observed at 4h. Further, compared to control animals, mice treated with EtOH+LPS had markedly increased hepatic neutrophil infiltration at both 4h and 24h, which was attenuated by RvE1 and RvD1 treatment. EtOH+LPS significantly induced expression of the hepatic neutrophil chemoattractants, Cxcl-1 and Cxcl-2, and pro-inflammatory cytokines, Tnf-α, Il-6, Il-1β, Il-18 at both 4h and 24h. Notably, RvD1, but not RvE1, significantly attenuated the EtOH+LPS-mediated Cxcl-1, Cxcl-2, Il-1β and Il-18 induction at 24h, suggesting distinct inflammatory pathway-specific actions of resolvins. In addition, RvD1 attenuated expression of the inflammasome components Caspase-1 and Asp, suggesting a suppressive effect of RvD1 on inflammasome activation.

Conclusions: Collectively, our data demonstrate that circulating and hepatic markers of inflammation were elevated in a time-dependent manner in liver injury induced by EtOH and LPS, with the peak inflammation observed at 4h compared to 24h post-LPS injection. Importantly, resolvin treatment significantly ameliorated inflammatory responses observed at 24h, and to a lesser extent at 4h, suggesting a potential pro-resolution effect, and thus may be a promising novel therapy for ALD.

A-336

Performance Evaluation of Anti-tTG IgA Assay on the Fully Automated BioCLIA®1200 Immunoassay Analyzer

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Background: Celiac disease (CD) is a life-long condition in which consumption of dietary gluten, leads to chronic inflammation and damage of the small intestinal mucosa. Tissue transglutaminases (tTG) has been identified as the major autoantigen in CD. IgA antibodies against tTG are highly disease specific serological markers for CD. Recently, the anti-tTG IgA assay coupling with the fully automated, random-access BioCLIA® 1200 chemiluminescent immunoassay system has been evaluated and launched. Methods: In this study, the analytical performances including dilution linearity, limit of detection (LOD), precision (intra-assay & inter-assay) of BioCLIA® anti-tTG IgA kit were evaluated, according to the CLSI guidelines. Total of 200 clinical samples, including 100 each of positive and negative samples from France, were both evaluated with a commercial anti-tTG IgA FEIA and BioCLIA® anti-tTG IgA kit. Furthermore, the various type of clinical samples including celiac disease (CD, N=10), autoimmune thyroid disease (N=50), type 1 diabetes (TIDM, N=50), rheumatoid arthritis (RA; N=50) and healthy donors (N=150) collected from local Chinese hospitals were also evaluated and analyzed. Results: The performance evaluation of HOB BioCLIA® anti-tTG IgA kit showed the accurate and faster results with an extended working range and good reproducibility. Compared with the FEIA kit, we observed the comparable sensitivity (99%; N=99/100) and specificity (98%; N=98/100) between two kits. The clinical sensitivity of CD were 90% (N=9/10), while the specificity for Chronic Diarrhea, Autoimmune Thyroid Disease, T1DM, RA, and healthy donors were 99.2% (N=129/130), 96% (N=48/50), 98% (N=47/49), 100% (N=50/50) and 99.3% (N=149/150), respectively. Conclusion: The anti-tTG IgA assay on the BioCLIA® 1200 automated platform exhibits an excellent sensitivity, a wider measurable range and shorter reaction time compared with traditional ELISA. It serves as a promising and environmental-friendly alternative for FEIA assay in the detection of CD autoantibodies.

A-337

New serological markers for celiac disease: Anti-neo-epitope human and microbial transglutaminases antibodies

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Objectives: Microbial transglutaminase (mTg) and human tissue tTG (tTg) complexed to gliadin peptides present neo-epitopes. Antibodies against these complexes are called tTg neo-epitope and mTg neo-epitope. Reliability of antibodies against the non-complexed and complexed forms of both transglutaminases to reflect intestinal damage and to diagnose the pediatric Celiac Disease (PCD) was compared.

Methods: 95 PCD patients, 99 normal children (NC) and 79 normal adults (NA) were tested using the following ELISAs detecting IgG or IgA or both IgA+IgG combined: tTg (for in house research use only), AESKULISA® tTg New Generation (tTg neo-epitope (tTg-neo)), AESKULISA® mTg (RDU) and AESKULISA® mTg neo-epitope (mTg-neo, RUO). Revised Marsh criteria were used for the degree of intestinal injury.

Results: All anti-mTg-neo and anti-tTg neo-epitopes were higher (p<0.001) compared to the single antigens. tTg-neo IgA and IgG/IgA were higher than mTg-neo IgA and IgA/IgG (p<0.0001). The antibody activities reflecting best the increased intestinal damage were: mTg-neo IgG > mTg-neo IgG > mTg-neo IgA > mTg-neo IgA/IgG. Taken together, mTg-neo IgG and mTg-neo IgA and IgA/IgG correlated best with intestinal pathology (r=0.5633, r=0.6165 & r= 0.6492; p<0.0001, p<0.0001 and p<0.0001, respectively).

Conclusion: The complexed forms of both transglutaminases exhibited a higher OD activity and better reflected intestinal damage in PCD, compared to the non-complexed forms. mTg is immunogenic in children with coeliac disease and by complexing to gliadin its immunogenicity and intestinal pathology reflection is enhanced.

A-338

Method comparison of AESKULISIDES ANCA for the diagnosis of ANCA-associated Vasculitis

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Background: AESKULISIDES ANCA is an indirect immunofluorescence assay used to detect anti-neutrophil cytoplasmic autoantibodies (ANCA) in human serum. This in vitro diagnostic assay is used as an aid for the diagnosis of ANCA-associated vasculitis (AAV) in conjunction with other clinical and laboratory findings. Methods: A method comparison of ethanol and formalin fixed granulocytes was carried out between AESKULISIDES ANCA (AESKU.Diagnostics) and the NOVA Lite ANCA of NOVA. 507 clinical serum samples (comprising 135 serum samples from patients with AAV and 375 samples from patients with other diseases) were analyzed by AESKULISIDES ANCA of AESKU.Diagnostics and the NOVA Lite ANCA of NOVA. Methods: A method comparison of ethanol and formalin fixed granulocytes was carried out between AESKULISIDES ANCA (AESKU.Diagnostics) and the NOVA Lite ANCA of NOVA. 507 clinical serum samples (comprising 135 serum samples from patients with AAV and 375 samples from patients with other diseases) were analyzed by AESKULISIDES ANCA of AESKU.Diagnostics and the NOVA Lite ANCA of NOVA. Results: In this cohort, AESKULISIDES ANCA Ethanol slides show higher sensitivities (48.5% vs. 36.4%) and specificities (69.3% vs. 55.2%) compared to NOVA. AESKULISIDES ANCA Formalin slides show higher sensitivities (50.0% vs. 37.9%) and similar specificities (90.7% vs 91.5%) compared to NOVA. Conclusions: AESKULISIDES ANCA Ethanol showed higher diagnostic sensitivity (48.5%) and specificity (69.3%) compared to the predicate assay NOVA Lite provided by NOVA (37.9%). However, the diagnostic sensitivity was comparable between the two (90.7% vs 91.5%).
Immunology

A-339

The prevalence of ASCA IgA and IgG antibodies is increased in manic bipolar disordered patients

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Objectives and study: anti-Saccharomyces cerevisiae antibodies (ASCA) are frequent in gastrointestinal inflammatory diseases. Given the concept of gut-brain axis and inflammation induced leaky gut syndrome in psychiatric settings, the prevalence of ASCA is of interest to be analyzed in bipolar disorder.

Aim: To study ASCA prevalence in bipolar disorder patients.

Methods: IgA+IgG (Check) ASCA were detected by ELISA (AESKULIPP/Stroh’s Check), in 170 bipolar patients, (52 depression and 118 manic), and compared to 69 healthy controls.

Results: We found that the prevalence of ASCA Check positivity was significantly higher in manic bipolar patients as compared to healthy controls (25% and 13% respectively p< 0.0023).

Conclusions: Increased prevalence of ASCA Check antibodies is found in manic phase of bipolar disorder. Environmental processed nutrients, enteric comorbidity or overall enhanced activated immune state can explain this high prevalence.

A-340

Performance evaluation of the specific proteins panel on the Alinity c system

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Background: Specific proteins are valuable markers for a variety of diseases including microbial infections, inflammatory response, cardiac risk, and even cancer. Abbott provides a broad spectrum of specific protein assays which enable diagnosis and management of many immune system related diseases. These assays include testing levels of the complement system to monitor inflammatory response and to help diagnose and monitor autoimmune diseases, such as rheumatoid arthritis. The Alinity ci system is part of a unified family of systems that are engineered for flexibility and efficiency. The design is based on insights from customers, resulting in a number of benefits including a smaller footprint, improved throughput, and greater with throughput with up to 1350 tests per hour. The Alinity ci system has an increased reagent load capacity, holding up to 70Clinical Chemistry reagents, onboard QC and calibrators, clot and bubble detection ability, and smartwash technology to provide consistent and reliable results.

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

Results: The observed results for precision, LoQ, method comparison, and defined measuring intervals for representative assays in the Specific Proteins Panel are shown in the table below.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Total %CV</th>
<th>LoQ</th>
<th>Method Comparison to ARCHITECT (Slope/r)</th>
<th>Measuring Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immuno-globulin A</td>
<td>≤ 1.8</td>
<td>3 mg/dl</td>
<td>0.98/1.00</td>
<td>5 mg/dl to 3850 mg/dL</td>
</tr>
<tr>
<td>Immuno-globulin G</td>
<td>≤ 1.5</td>
<td>10 mg/dl</td>
<td>0.99/1.00</td>
<td>320 mg/dl to 4675 mg/dL</td>
</tr>
<tr>
<td>Immuno-globulin M</td>
<td>≤ 3.2</td>
<td>5 mg/dl</td>
<td>1.02/1.00</td>
<td>5 mg/dl to 1815 mg/dL</td>
</tr>
<tr>
<td>Prealbumin</td>
<td>≤ 2.2</td>
<td>1.0 mg/dl</td>
<td>1.00/1.00</td>
<td>3 mg/dl to 66 mg/dL</td>
</tr>
<tr>
<td>Complement C3</td>
<td>≤ 1.8</td>
<td>5 mg/dl</td>
<td>1.03/1.00</td>
<td>11 mg/dl to 385 mg/dL</td>
</tr>
<tr>
<td>Complement C4</td>
<td>≤ 1.7</td>
<td>0.7 mg/dl</td>
<td>0.98/1.00</td>
<td>2.9 mg/dl to 72.0 mg/dL</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>≤ 1.5</td>
<td>3 mg/dl</td>
<td>0.99/1.00</td>
<td>8 mg/dl to 300 mg/dL</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>≤ 1.4</td>
<td>3 mg/dl</td>
<td>1.02/1.00</td>
<td>16 to 310 mg/dL</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>≤ 3.7</td>
<td>3 mg/dl</td>
<td>0.98/1.00</td>
<td>11 to 240 mg/dl</td>
</tr>
</tbody>
</table>

Conclusion: Representative clinical chemistry assays utilizing photometric technology on the Alinity c system demonstrated acceptable performance for precision, sensitivity, and linearity. Method comparison data showed excellent agreement with on-market ARCHITECT clinical chemistry assays.

A-341

Laboratory biomarkers in the monitoring of a patient with multiple myeloma

J. L. García de Veas Silva, M. Lopez Velez, T. De Haro Romero, A. Espuch Oliver, J. Garcia Lario, T. De Haro Muñoz. Hospital Universitario Campus de la Salud, Granada, Spain

Background: Multiple Myeloma (MM) is a malignancy of B cells characterized by an atypical proliferation of plasm cells. IgD MM has a very low incidence (2% of MM cases) and it’s characterized by an aggressive course and a worse prognosis than other subtypes. The free light chains in serum (FLC) are very important markers for monitoring patients with multiple myeloma (MM) and other monoclonal gammopathies. When the serum FLCs are present in low concentrations, they are difficult for the detection by conventional methods as serum protein electrophoresis (SPE) and immunofixation (IFE). We report the case of a patient where FLCs are either undetectable or barely detectable using the conventional qualitative assays.

Case report: A 50 years old man was diagnosed in June 2011 of IgD Kappa multiple myeloma with primary amyloidosis associated. He began treatment with VAD (vincristine, doxorubicin and dexamethasone) and hemodialysis. He received three cycles of VAD from July 2011 to August 2011 but the κ/λ FLC ratio was altered during this treatment (from an initial value of 1570 mg/L in July to a value of 1633 mg/L in August). The IFE was positive (IgD Kappa) during the treatment. Due to the minimum response of the disease and the development of demyelinating neuropathy, the treatment was changed to bortezomib and dexamethasone. Then, the patient received eight cycles from September 2011 to April 2012 with a normalization of the κ/λ FLC ratio from an initial value of 1579 mg/L in September to a value of 1.62 mg/L at the end of March 2012 with negative IFE. The patient’s condition improved with this treatment and achieved the complete remission (CR). Three months later, the κ/λ FLC ratio began to increase predicting a relapse with a value of 2.52 mg/L in July, 4.27 mg/L in August, 60.23 mg/L in October and a maximum value of 135.85 mg/L in December.

In these months, the IFE was normal. In January 2013, the κ/λ FLC ratio remained altered (97.41 mg/L) and the IFE was positive (IgD Kappa) for first time in the relapse.

Conclusions: This case is a good example of the utility of κ/λ FLC ratio in the monitoring of multiple myeloma. The κ/λ FLC ratio can detect when the chemotherapy applied isn’t completely effective or it can predict future relapses in the patient.
Laboratory biomarkers in the identification of residual disease in patients with multiple myeloma

J. L. García de Veas Silva, M. Lopez Velez, A. Espuch Oliver, T. De Haro Romero, J. García Lario, T. De Haro Muñoz, Hospital Universitario Campus de la Salud, Granada, Spain

Background: The quantification of heavy/light chains pairs (HLC) by the new immunossay Hevylite is based on the recognition of epitopes spanning the junction of the immunoglobulin’s heavy and light chains. This assay can identify separately the different light chain types of each immunoglobulin class: IgGk, IgGL, IgAK, IgAL, IgMK, and IgML. Of particular interest and novelty is the possibility to quantify separately both isotypes of the tumor related immunoglobulin. In this clinical case we show the utility of the quantification of HLC IgAK, IgAL and IgAK/IgAL ratio as a method to monitor and identifying residual disease in a patient with IgA-Kappa Multiple Myeloma.

Case presentation: We present the case of 51 years old man diagnosed of IgAK Multiple Myeloma ISS III stage [hypercalcemia (16.6 mg/dl), increased IgA (4449 mg/dl) and total proteins (12.6 g/dl), norrmonic anemia (9.5 g/dl of hemoglobin), altered ratio of serum free light chains (free kappa=219 mg/dl, free lambda=1.01 mg/dl, ratio=216.83) and osteolytic bone lesions (punched-out lesions in skull and vertebral compression)]. At diagnosis (Day 0) the serum proteinogram (SPE) shows a well-defined monoclonal large peak in the gamma region (4.34 g/dl correspond to monoclonal component) identified by immunofixation as IgA-Kappa. The IgA HLC ratio (IgAK=66.664 g/l, IgAL=6.302 g/l, ratio=10.57) identified clonal disease IgA- Kappa at diagnosis. The patient began treatment with Bortezomib, Cyclophosphamide and Dexamethasone and the monoclonal protein was monitored by SPE, IFE and HLC. During the treatment, the monoclonal protein was decreasing with reduction of the peak in SPE and the HLC ratio remained altered confirming the presence of the monoclonal protein. The monoclonal component IgA-Kappa was decreasing due to the good response to the treatment. At day +58 (after 4th cycle of chemotheraphy) there was a little peak in SPE (0.18 g/dl of monoclonal component), with positive IFE and altered ratio HLC (IgAK=3.566 g/l, IgAL=0.664 g/l, ratio=5.37). At day +68 the SPE was negative but the HLC ratio remained altered (IgAK=3.566 g/l, IgAL=0.664 g/l and ratio=5.37) confirming the existence of monoclonal protein that it was verified by IFE. At day +131 (end of 5th cycle of chemotheraphy) the SPE, HLC and IFE were negative confirming the absence of monoclonal protein due to the good response to the treatment. At the end of the treatment (day +184) after six cycles of chemotherapy the patient achieved a status of complete remission with negative immunofixation, ~5% of plasma cells in bone marrow and normal HLC pairs ratio.

Conclusion: The monitoring of IgA MM requires the measures of SPEP, IFE and total IgA. The use of the HLC IgAK, IgAL and their ratio IgAK/IgAL presents itself as an alternative method with high sensitivity for monitoring these patients, particularly in situations where traditional techniques show limitations (e.g. low concentrations, interference of other serum proteins, strong polyclonal background). The high sensitivity of the determination of HLC allows typing monoclonal component providing equivalent information to the immunofixation, with the added value of reporting a quantitative value.

Diagnostic Utility of Antitransglutaminase Antibodies IgA in the study of Celiac Disease

J. F. Cuadros-Muñoz, M. Mayor-Reyes, J. D. Santortoribio, S. Pérez-Ramos, C. Cañavate-Solano. Hospital Universitario Puerto Real, Puerto Real, Spain

Celiac disease (CD) is an autoimmune disease caused by a gluten proteins intolerance and manifested in individuals with genetic predisposition when there is an intake of foods rich in gluten such as wheat, barley or rye. This disease occurs with severe atrophy of the mucosa of the upper small intestine and the only treatment currently available consists of a gluten-free diet that must be strictly maintained. Currently, in clinical laboratories, the study of CD is usually initiated with the determination in serum of anti-tissue transglutaminase IgA antibodies (t-TG IgA) and/or IgG (t-TG IgG). These techniques present a series of advantages such as complete automation and high sensitivity and specificity, which have displaced other serological tests such as anti-endomysial antibodies and anti-gliadin antibodies as the technique of choice. However, a diagnosis of celiac disease should not be based only on a positive result of anti-t-TG IgA but should be based on the evaluation of the set of clinical and laboratory data available, mainly, the small bowel biopsy that remains the test “gold standard” for the diagnosis of CD. The main purpose of this study was to analyze the results of t-TG IgA antibodies in our laboratory and compare them with the diagnoses of patients to check how many of those positive results of anti t-TG IgA were confirmed or not with a diagnosis of CD. The samples used were serum from patients who the t-TG IgA antibodies was requested for a period of 8 months in 2017. Samples were processed in the ZENIT RIA autoanalyzer (A.MENARINI diagnostics) by chemiluminescent immunoassay (CLIA). A value greater than 10 AU/ml was considered a positive result. Next, we reviewed the clinical histories of those patients whose results were positive to verify the definitive diagnoses. In this period of time, a total of 3040 determinations of anti-tTG IgA antibodies were made by CLIA, of which 74 (2.4%) were positive. Of these 74 patients, 50 (67.6%) were diagnosed with CD, 18 patients (24.3%) with probable diagnosis/suspicion of CD, and 14 (19.0%) who were not diagnosed with CD. Definitely, anti-tTG IgA antibodies have a high sensitivity for screening in the study of CD because in this study, of the total number of patients with positive results, 81.1% were confirmed with a definitive or probable diagnosis of CD.

Analysis of the Chemiluminescent Immunомassay as a method of screening in the study of Systemic Autoimmune diseases

J. F. Cuadros-Muñoz, M. Mayor-Reyes, J. D. Santortoribio, S. Pérez-Ramos, C. Cañavate-Solano. Hospital Universitario Puerto Real, Puerto Real, Spain

Systemic autoimmune diseases (EAS) are a group of diseases with extensive clinical expression, chronic course, among which are systemic lupus erythematosus (SLE), polymyositis / dermatomyositis (PM / DM), mixed connective tissue disease (EMTC), sjogren’s syndrome (SS) or systemic sclerosis (ES). In the study of EAS, the determination in serum of antinuclear antibodies (ANA) is usually used as the initial screening test. ANA are a heterogeneous group of antibodies developed by the immune system directed against a large variety of antigens located in the nucleus and cytoplasm of cells of different organs and body tissues. In our laboratory, the determination of ANA is carried out using the chemiluminescence technique (CLIA). However, due to the false positives presented by this method, when a positive result is obtained by CLIA, confirmation by indirect immunofluorescence (IF) on human epithelial (HEp-2) cells is required, which is the “gold standard” test. However, the IFI has disadvantages that are mainly its subjectivity and a higher cost. The aim of this study was to calculate the positive predictive value of the CLIA technique, comparing it with the IFI to evaluate its usefulness in the screening of EAS. The results obtained during the second semester of 2017 were collected in serum from patients who were asked to determine ANA. Samples were processed by CLIA in the LIAISON® Analyzer (Diasorin Palesx Medical). A sample with a value equal to or greater than the 1.5 index was considered a positive result. In total, 2,875 samples were processed, of which a positive value was obtained in 312 (10.9% of the total). These 312 samples were analyzed by IFI using the Olympus CX41 fluorescence microscope. Titrations with IFIs less than 1:40 were considered negative and those greater than or equal to 1:40 were positive. A total of 198 positive results (63.5%) and 114 negative results (36.5%) were obtained by IFI. In the positive results there were titrations from 1/40 to 1/1280 and with the following distribution of patterns:

• SPECKLED: 64
• RETICULAR/MITOCHONDRIAL-LIKE:32
• CENTROMERE:32
• HOMOGENEOUS:20
• CYTOPLASMIC:20
• NUCLEAR:10
• CYTOPLASMIC AND SPECKLED:4
• CYTOPLASMIC AND NUCLEAR DOTS:4
• CYTOPLASMIC AND NUCLEAR:2
• NUCLEAR DOTS:2
• HOMOGENEOUS AND SPECKLED:2
• P.C:N:2
• NUCLEAR AND NUCLEAR DOTS:2
• RETICULAR/MITOCHONDRIAL-LIKE AND NUCLEAR DOTS:2

With these results, the positive predictive value of the CLIA technique obtained was 63.5%. This confirmed one of the disadvantages of chemiluminescence in the initial screening of EAS, which was the presence of false positives. In conclusion, the CLIA technique is useful as a method of scrutiny in the study of EAS because it is an automated, objective and highly sensitive technique. However, before a positive result this must be confirmed by IFI.
Necrotizing myopathy caused by combination of antisynthetase syndrome and mycobacterial infection non detected by Quantiferon: Diagnosis by laboratory methods.

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Background: Antisynthetase syndrome is an inflammatory muscle disease related to dermatomyositis and polymyositis. The hallmark of antisyhtetase syndrome is the presence of serum autoantibodies directed against aminoacyl-tRNA synthetases. These are cellular enzymes involved in protein synthesis. Individuals with antisynthetase syndrome and other autoimmune disorders are also at increased risk of infection, both due to immune compromise from the disease itself and due to immunosuppressive therapy. Moreover, false negative or indeterminate Quantiferon results can be seen in elderly, immunocompromised, chronic and severely diseased patients.

Methods: A 62 year old Afro-Caribbean woman with a past medical history of diabetes mellitus type II with recent travel to Trinidad arrived to our institution with fever and left upper extremity muscle pain. A prior muscle biopsy showed necrotizing myopathy, for which she had a 4-month course of steroids treatment. Chest CT scan demonstrated heterogeneous appearance of the left pectoral muscle with surrounding fluid attenuation, subcutaneous fat infiltration and scattered subcentimeter reactive lymph nodes. Relevant labs include bandemia, elevated procalcitonin, liver function tests (LFT) and creatine phosphokinase (CPK). An extensive work-up was done for autoimmune disease and myositis. Myositis panel assay, a test that detects serum markers for myositis using Western Blotting, which includes EJ antibody, RNP, Mi-2, Ku, Signal Recognition Particle (SRP), Mi-2, PL-7, PL-12, and OJ antibodies was performed. HTLV1 and 2 antibodies and Quantiferon test, were also performed.

Results: Myositis panel assay was positive for EJ antibody, and negative for RNP, Mi-2, Ku, Signal Recognition Particle (SRP), Mi-2, PL-7, PL-12, and OJ antibodies. Multiprobe flow immunoassay was negative for Proteinase-3, Myeloperoxidase, ANA, Scleroderma, B2-glycoprotein and anti-smith antibodies. Chemiluminescent immunoassay detected HTLV1 and 2 antibodies in this patient, but the immunoblot was negative for the HTLV-1 and -2 viruses. Quantiferon for tuberculosis was indeterminate. The patient decamped and expired on the 9th day of the hospitalization. During the hospitalization, questions were raised as to whether the patient had an autoimmune myositis versus other causes of necrotizing myopathy. At autopsy, chronic myopathic pathology with muscle atrophy and fibrosis was seen. In addition, acute abscesses with areas of necrotizing inflammation were identified in chest wall, lymph node, adipose, muscle and liver tissue, showing numerous acid-fast bacilli on FITE stain. Interstitial lung disease, interstitial inflammation and fibrosis were also present. Positive mycobacterium tuberculosis complex was identified by molecular detection on Paraffin.

Conclusion: Our case illustrates the challenging diagnostic process of Antisynthetase syndrome in a patient with concurrent mycobacterial infection with tropism for soft tissue. Furthermore, patients who are immunocompromised and have positive anti EJ antibodies may have unreliable Quantiferon test results. Alternative approaches should be sought for determining patient’s myocorticosteria status in these clinical settings.

Performance Evaluation of Anti-Proteinase 3 IgG Antibody Assay on the BioCLIA® 1200 Automated Chemiluminescence Immunoassay Analyzer

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Background: Anti-neutrophil cytoplasmic antibody (ANCA) testing has revolutionized the diagnosis and treatment of the various autoimmune mediated vasculitis. The PANCA and cANCA auto-antibodies have been proven to be useful clinically and interesting scientifically for the detection of diseases such as Wegener’s granulomatosis (WG), crescentic glomerulonephritis, polyarteritis nodosa and Churg-Strauss syndrome. Anti-proteinase 3 (PR3) IgG antibodies is one of the primary cANCA present in patients with WG and the specificity of anti-PR3 antibodies for WG was determined to be 97% Recently, the innovative HOB BioCLIA™ anti-PR3 assay, 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™ anti-PR3 assay according to the CLSI guidelines. Furthermore, total of 100 clinical samples with indirect immunofluorescence assay (IFA) results, were analyzed by both BioCLIA™ and ELISA (from an international ally renowned manufacture). Sensitivity, specificity and total agreement of the compared assays were analyzed. Lastly, a total of 240 clinically characterized samples were used to study clinical sensitivity and specificity, including 40 patients for WG, 50 patients for Systemic Lupus Erythematosus (SLE), 50 patients for Rheumatoid Arthritis (RA), and 100 samples for healthy donors from local Chinese hospitals.

Results: The BioCLIA™ anti-PR3 assay performed good linearity ranging from 2-400 RU/mL and the LOD was 0.17 RU/mL. In the precision study, the CV% was 3.98% for intra-assay and 5.24% for inter-assay, respectively. Bilineub (up to 50 mg/dL), hemoglobin (up to 400 mg/dL), lipid (up to 2000 mg/dL), RF (up to 1000 IU/mL) and HAMA (up to 200 mg/dL) did not affect the detection of anti-PR3 IgG in serum. In a clinical evaluation, using 100 clinical samples with IFA assay confirmed results, we found the BioCLIA™ has higher sensitivity of 95.0% (57/60) than ELISA result of 91.7% (55/60), but with a similar specificity of 98.5%. The total agreement of BioCLIA™ & ELISA compared to IFA assay were 96.0% (96/100) & 94.0% (94/100), respectively. From the clinical study in Chinese patients, the positive rate showed on BioCLIA™ anti-PR3 assay in WG, SLE, RA and healthy donors were 95.0% (38/40), 6.0% (3/50), 1.6% (1/50) and 1.0% (1/100), respectively.

Conclusion: BioCLIA™ anti-PR3 assay is an innovative semi-quantitative assay which exhibits fast and accurate analysis and demonstrates linearity in an extended analytical measuring range as well as good reproducibility. In the aspect of clinical comparison, the kit offered a better clinical relevance when compared with ELISA, and a good agreement with IFA assay, which is considered as the gold standard method. It serves as a promising and fully automated alternative for IFA assay in the detection of anti-PR3 IgG antibodies and valuable to aid in the diagnosis of WG.
Presence of anti-cN-1A (Mup44, NT5c1A) IgG is specific for Sporadic Inclusion Body Myositis

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Background: Sporadic Inclusion Body Myositis (sIBM) is an autoimmune disease manifesting with muscle degeneration, inflammatory infiltrates and inclusion vacuoles. Diagnosis of sIBM is hampered by its imprecise characteristics, at times indistinguishable from other Idiopathic Inflammatory Myopathies, but may now be assisted by detection of sIBM-specific autoantibodies targeting muscle antigen Mup44, identified as cytosolic 5’-nucleotidase type 1A (cN-1A; Mup44; NT5c1A). This study evaluated sensitivity and specificity of an anti-cN-1A IgG serological assay in sera from patients with and without sIBM.

Methods: Serum from patients with clinically and pathologically diagnosed sIBM (n=68), suspected sIBM (n=15), myositis controls [including dermatomyositis (n=4), polymyositis (n=7); unspecified myositis without sIBM (n=94), muscle atrophy (n=1), myonecrosis (n=4)], from patients with SLE (n=33), scleroderma (n=20), Sjögren’s (n=20), rheumatoid arthritis (n=20) and from healthy controls (n=254) were tested for anti-cN-1A IgG using an anti-cN-1A ELISA (full-length antigen, Euroimmun AG). Percent agreements were 83.3% (κ, 0.661) and 84.9% (κ, 0.671) between PROTIA Allergy-Q and ImmunoCAP (results from one Helena, two Siemens). Laboratories blindly analyzed samples according to their SPEP standard operating procedures. Three laboratories used agarose gel electrophoresis (AGE) (one Helena, two Siemens) and one used capillary electrophoresis accuracy study

Conclusions: The presence of anti-cN-1A in serum appears to be disease-specific for sIBM. These antibodies are found at a moderate prevalence, but are only rarely detected in other autoimmune conditions. Thus, anti-cN-1A ELISA may support the diagnosis of sIBM and accelerates the suspected diagnosis in cases of positivity, where muscle biopsy is delayed or unfeasible.

Comparison of two multiple allergen simultaneous tests: AdvanSure Alloscreen and PROTIA Allergy-Q

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Background: Multiple allergen simultaneous test (MAST) has been widely performed as a screening tool for detecting allergen specific immunoglobulin (Ig) E. However, since MAST has no standard reference method, comparisons with existing assays would be practical to assess the performance of the new one. Recently, PROTIA Allergy-Q (ProteomeTech, Seoul, Korea), a new automated analyzer with high-throughput for MAST, has been introduced in South Korea. In this study, we compared the performance of PROTIA Allergy-Q with MAST assay currently utilized to evaluate its usefulness in clinical laboratories.

Methods: Sixty serum samples with positive results in AdvanSure Alloscreen (LG Life Sciences, Seoul, Korea) (30 for food and 30 for inhalant) were subjected to PROTIA Allergy-Q. We assessed positivity rates and percent agreements according to allergen panel or each allergen. 267 ImmunoCAP test (Phadia, Upssala, Sweden) were repeated with sera demonstrating discrepancies between two MAST assays.

Results: The positivity rates of inhalant panel and food panel were 35.8% and 31.2% by PROTIA Allergy-Q, 36.8% and 33.5% by AdvanSure Alloscreen, respectively. Percent agreements were 83.3% (κ, 0.661) and 84.9% (κ, 0.671) between PROTIA Allergy-Q and AdvanSure Alloscreen. However, 19 allergens in inhalant panel and 13 allergens in food panel showed discordant rates below 80% ranging from 46.7% to 76.7%. The agreement of PROTIA Allergy-Q and ImmunoCAP (69.3%) results was superior to one of AdvanSure Alloscreen with ImmunoCAP (47.9%) results.

Conclusion: Our study demonstrated that PROTIA Allergy-Q showed a good agreement with current MAST assay and better agreement with ImmunoCAP assay than current MAST assay.
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Study of the frequency of test positivity for Arboviruses in a 5-year period in the city of São Paulo

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Background: Arboviruses represent a growing problem for the propagation potential characterized by ease adaptability in hosts. The chances of epidemics are extensive due to universal susceptibility and their numbers are worrisome due to the presence of large numbers of severe and fatal cases, or with neurological, articular and hemorrhagic involvement. In the Brazilian epidemiological context, the highest circulating arboviruses are dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV), although there are others with potential for dissemination in the country. This framework requires coping with broad-spectrum policies and interventions involving all public health, especially the need for efficient and rapid laboratory tests that diagnose and monitor patients. In this study the authors aimed to measure the number of positive cases by the available tests and the number of requests for arboviruses: DENV, CHIKV and ZIKV, in the last 5 years in São Paulo. Methods - Results of requests for DENV, CHIKV and ZIKV tests, of 5 years (2013 to 2017), were analyzed in the database of a large laboratory in the city of São Paulo. The study was retrospective and observational. The tests were performed using the following methods: DENV-ELISA) - Panio Focus Novagnost, Euroimmun, Siemens®, Immunochromatography (WAMA®), CHIKV-Immunochromatography - OrangeLife and PCR In real time. Results: From the total of 59793 arboviruses test requests for the studied period, 26617 positive cases were shown represented in the several tests in Table 1. Conclusion: Based on the data found, that as the years passed, the types of arboviruses increased. Until 2015, there was practically no request for tests for CHIKV or for ZIKV. From 2016 these last arboviruses began to present positivity. We conclude that arboviruses are emerging in Brazil and that several diseases, such as Yellow Fever, may appear.

Table 1. Representation of the positive cases for arboviruses in the different tests used in laboratory routine.

<table>
<thead>
<tr>
<th></th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
<th>2017</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td>DENV-1gM</td>
<td>0</td>
<td>1</td>
<td>1121</td>
<td>608</td>
<td>376</td>
<td>2173</td>
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<tr>
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<td>0</td>
<td>15.18</td>
<td>47.28</td>
<td>37.51</td>
<td>645</td>
</tr>
<tr>
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<td>5790</td>
<td>1802</td>
<td>4916</td>
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<td>22893</td>
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<td>4.32</td>
<td>22893</td>
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<tr>
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<td>22</td>
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<td>216</td>
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<td>0</td>
<td>0</td>
<td>559</td>
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</tr>
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</table>

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Performance Evaluation of the Newly Developed HOB BioLine® Pro Automated Allergen-Screen Assay

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Background: A new multiple allergen simultaneous test on the immunoblot has recently been developed that is simple, rapid, and economical, and requires a small amount of serum samples. The BioLine® Allergy Screen assay is an advanced multiplex test that allows for simultaneous detection of specific IgE (sIgE) against multiple allergens on the fully automated instrument including the sample addition and data interpretation. In this newly developed assay, specific IgE from the patient sample is captured by the allergen coated to the nitrocellulose strip. After washing, the biotinylated anti-IgE is added and incubated in the trap. Excess biotinylated anti-IgE is removed by the next wash step and then streptavidin labeled with alkaline phosphatase is added. It forms the complexes consisting of allergen/ specific IgE/ biotinylated anti-IgE/streptavidin conjugate. The color purple is developed on the strip by the addition of substrate solution. The test results are recorded after air drying and image scanned by an integrated camera automatically. We evaluated the specific antigen detection results from multiple allergen simultaneous tests between ImmunoCAP and BioLine® Pro systems. Methods: In this study, the analytical performance of Allergen-specific IgE to 10 inhalant allergens including D1 (Dermatophagoides pteronyssinus), D2 (Dermapthagoides farinae), E1 (Cat epithelium), E5 (Dog dander), H1 (House dust), M6 (Alternaria alternata), T3 (Common silver birch), W1 (Common ragweed), W21 (Wall pellitory), W6 (Mugwort) and 10 food allergens including F14 (Soybean), F20 (Almond), F202 (Cashew nut), F27 (Beef), F3 (Codfish), F31 (Carrot), F5 (Rye), F84 (Flour), F85 (Celery), F95 (Peach). The procedure was evaluated in parallel and sensitivity according to the CLSI guideline. The Clinical Laboratory Evaluation of 10 clinical samples was evaluated by both of HOB BioLine® Pro Allergy Screen assay and ImmunoCAP systems. Results: The CVA% of D1, D2, E1, E5, H1, M6, T3, W1, W21, W6, F14, F20, F202, F27, M3, F31, F5, F84, F85 and F95 were in a range from 4.47–7.87% for within-run, and 5.58–8.35% for total-run. Bilirubin (up to 20 mg/dL), hemoglobin (up to 40 mg/dL), and lipid (up to 2000 mg/dL) did not affect the IgE qualitative detection in serum.

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Optimization of resources in the clinical laboratory: Impact in the safety of the patients

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Introduction: The Clinical Laboratory is in permanent evolution and should not be limited to providing results as a data factory. The results should be in

niques and protocols should be applied to help the clinician to diagnose the patients in the shortest time. The introduction of comments that guide the clini

icians and the use of additional tests in patients with unexpected analyti

cal findings will allow the clinicians to take the correct diagnostic decisions. Case presentation: A six-year-old woman was admitted to the emergency services due to heavy bleeding after tooth extraction. She reported recurrent hemorrhages since the dental intervention together with fatigue and dyspnea. Furthermore, the patient presented back bone pain. The following parameters were altered in the laboratory report: Hemoglobin=8.7 g/dL, calcium=11.15 mg/dL and total serum protein=13.70 g/dL. The patient was referred to her primary physician to study the source of the anemia. A hyperproteinemia (13.2 g/dL) was detected in the Laboratory accompanied with anemia (7.8 g/dL) and hypercalcemia (11.15 mg/dL). Furthermore, a marked Rouleaux phenomenon is observed in the peripheral blood smear. With these clinical findings associated with multiple myeloma (MM), the protocol established for the screening of a monoclonal gammopathy (MG) is applied. In the serum protein electrophoresis was observed a well-defined monoclonal peak of 6.9 g/dL. The immunoglobulin levels were IgA=6856 mg/dL, IgG=198 mg/dL and IgM=6 mg/dL. The large increase in IgA levels was associated to a marked decrease in IgG and IgM levels. According to our protocol, serum free light chains (FLC) were quantified. A K/L ratio of 0.03 was obtained showing lambda monoclonality (Kappa=4.93 g/dL, Lambda=170.26 mg/L). Following up the protocol, a serum immunofixation was performed identifying the monoclonal component as IgA-Lambda. With these findings, the clinical case was presented in the Unit of Monoclonal Gammopathies (UGAM) of the Hospital to inform to the clinicians. The UGAM is a multidisciplinary group formed by specialists from different clinical specialties involved in the study of patients with MG (Clinical Biochemistry, Hematology, Nephrology, Pathology, Cardiology, Osteoporosis, Neurology and Rheumatology). The patient is referred to the Hematology Unit to complete the study. A bone marrow study showed a 10% of plasma cells with pathological phenotype (98.9% with lambda clonality) and several bone lesions were observed in the PET-CT scan. With these clinical data the patient was diagnosed of IgA-lambda MM Stage II by the hematologist. Conclusions: The laboratory has a essential role in the diagnosis of patients with MG. In the context of clinical symptoms associated to MG (bone pain, pathological fractures, anemia, and hyperproteinemia), the application of this protocol allowed us to identify the presence of a monoclonal component in the blood. The quick communication of the results to the clinicians at the UGAM meeting helped a rapid diagnosis of the patient. Communication between professionals from different clinical units is essential in daily practice for a correct interpretation and proper use of laboratory findings. Patient safety must be a priority in the Clinical Laboratory.
Measurement of C1 inhibitor protein using the Optilite® automated turbidimetric analyser


C1-INH (C1 inactivator / C1 inhibitor) is a protease inhibitor which controls activation of the classical complement pathway, fibrinolytic, clotting, and kinin pathways. The Optilite® C1 inactivator kit is intended for the quantitative in vitro measurement of C1-INH in serum, lithium heparin and EDTA plasma using the Binding Site Optilite analyser. C1-INH deficiency causes an increase in bradykinin, leading to vasodilatation, fluid extravasation and ultimately angioedema, and can be hereditary (HAE) or acquired (AAE). It is important to distinguish between angioedema related to C1-INH and that caused by other mechanisms, as treatment options are different. In both HAE and AAE, C1-INH protein concentration is used alongside C1-INH functional tests and C4 concentration to aid in patient diagnosis. Here we describe a fully automated assay for the measurement of C1-INH protein concentration on the Optilite turbidimetric analyser. A linear study was performed based on CLSI guideline EP6-A. The linearity of this assay was confirmed over 0.07 - 0.47 g/L at the standard 1+4 analyser dilution. A precision study based on CLSI guideline EP05-A2 was performed over 21 days. 5 samples with different C1-INH concentrations (0.12-0.41 g/L) were run in duplicate, with two runs per day using 3 reagent lots and 5 different analysers. The within run, between run, between day, between batch and between instrument percentage coefficients of variation (%CVs) were all <6%. The total %CV was <8% in all 5 samples. A 95th percentile reference interval of 0.21-0.38 g/L was generated by measuring C1-INH in serum samples from 120 healthy adult blood donors (median 57 years; range 23-94). A comparison of C1-INH concentration was made between the Optilite assay and a clinically used predicate assay using 260 clinical samples. There was a strong correlation between the assays (R^2=0.94, p<0.0001, Passing and Bablok slope y=0.83x), with 99.6% agreement in determining whether samples were above or below the lower limit of the normal range. In conclusion, the Optilite C1 inactivator assay allows the automated and precise quantification of C1-INH concentrations in patient sera samples and it correlates well with existing methods. It could therefore be used as a tool to aid in the investigation of the cause of angioedema.

A-356

Anti-parietal cell antibodies a frontline marker in Primary Care

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Background: There is a high prevalence of vitamin B12 deficiency. Anti-parietal cell antibodies (APCA) and/or intrinsic factor blocking antibodies (IFBA) can cause pernicious anemia (AA) which increases the risk of gastric carcinoma. The aim is to study the frequency of APCA and IFBA in primary care patients with severe vitamin B12 deficit, and to correlate with demographic characteristics and hematological values.

Methods: An observational study was designed and conducted from 1st May to 30th September 2017 in a community University Hospital covering a Health Department (HD). Participants were Primary care patients of the HD. In consensus between the laboratory and General Practitioners (GP’s) an intervention was designed that consisted in the laboratory information system automatically registering APCA and IFBA to the laboratory request of any primary care patient with a new s-vitamin B12 < 73.8 pmol/L (severe vitamin B12 deficit). We studied the number of patients with APCA and/or IFBA positivity, and their demographic data, MCV and haemoglobin values. APCA and IFBA were performed using the EliA immunoassay on the Phadia 2500 system according to the manufacturer’s instructions (Phadia GmbH, Freiburg, Germany).

Results: There were 77 new cases of severe vitamin B12 deficit. Among those, 44 (57.1%) were APCA+, and 11 (14.3%) were IFBA+. Age and sex did not significantly differ among patients with positive or negative antibodies. Table shows percentage of patients showing anemia or macrocytosis regarding the results of APCA and IFBA.

<table>
<thead>
<tr>
<th>ANEMIA</th>
<th>MCV</th>
<th>APCA</th>
<th>IFBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO</td>
<td>YES</td>
<td>&lt;100IL</td>
<td>&gt;100IL</td>
</tr>
<tr>
<td>Positive 19 (36.5%)</td>
<td>14 (56.0%)</td>
<td>21 (40.4%)</td>
<td>12 (48%)</td>
</tr>
<tr>
<td>Positive 33 (63.5%)</td>
<td>11 (44.0%)</td>
<td>31 (59.6%)</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>Positive 43 (82.7%)</td>
<td>23 (92.0%)</td>
<td>46 (88.5%)</td>
<td>20 (80.0%)</td>
</tr>
<tr>
<td>Positive 9 (17.3%)</td>
<td>2 (8.0%)</td>
<td>6 (11.5%)</td>
<td>5 (20.0%)</td>
</tr>
</tbody>
</table>

Conclusion: The automated strategy to identify subjects with APCA+ and or IFBA+ seemed successful given they efficiently detected APCA positivity in more than half of patients with severe vitamin B12 deficiency. The positivity would diagnose AAG and indirectly would confirm vitamin B12 deficit without confirmatory tests, turning APCA into a frontline marker in primary care.

A-357

Performance characteristics of the VaccZyme Salmonella Typhi Vi IgG commercial ELISA

L. Williams, S. Harding, A. Parker, The Binding Site Group Ltd, Birmingham, United Kingdom

Background: Antigen-specific serum IgG measurements are used to assess immune system competence and recovery, and may be used to support treatment decisions. Evaluation of adaptive immunity requires initiating B cell stimulation with both protein and polysaccharide vaccines. The current gold standard polysaccharide vaccine used for assessment is Pneumovac®/23 but interpretation can be complicated. Typhim Vi® is a Vi capsular polysaccharide vaccine administered to populations at risk of typhoid fever, i.e. in areas of endemic typhoid fever or to individuals travelling to such areas. Recent reports suggest that measurement of the IgG response to Typhim Vi may have utility in supporting a diagnosis of antibody deficiency. Typhim Vi has now been included in the recommendations for use as a diagnostic vaccine. Here we describe the performance of the VaccZyme™ human anti-Salmonella typhi Vi IgG ELISA which has been developed for the measurement of typhi Vi IgG. The performance characteristics from the production of six consecutive different kit batches are reported.

Methods: The concentration of Typhim Vi IgG was measured using the VaccZyme human anti-Salmonella typhi Vi IgG ELISA (The Binding Site Group Ltd., Birmingham, UK) in serum samples obtained from 40 adult blood donors (15 male and 25 female, aged 18-66 years). The measuring range of the assay was 7.4-6000/mL. Results: Variation in coating of the microtitre plates with Typhim Vi® was assessed. Median coefficient of variation (CV) for coating each individual batch was <5%. Median CV from six independent batches was 4% (n=24; range 3.3-5%). Target concentration recoveries of 10 samples were assessed for each batch. The percentage recoveries for typhi Vi IgG concentrations ranged from 96-105%. Typhim Vi IgG concentration comparisons between the different batches were performed using 30 serum samples. A median Passing Bablok regression of 1.05 (range 0.97-1.11) was obtained with a median linear regression correlation coefficient of r=0.99 (range 0.97-0.99). A precision study was performed for each of the six batches using 10 samples with analyte concentrations between 11.5-433 U/mL. The between run coefficients of variation (CVs) were 5-10% for all samples.

Conclusion: The VaccZyme anti-Salmonella typhi Vi ELISA provides a reliable and precise method for quantifying Typhi Vi IgG in human serum with high batch-to-batch reproducibility.

A-358

Evaluation of Two Fecal Calprotectin Assays

X. Zhang, K. Bowers, J. Andrusko, S. Richter, T. M. Daly, Cleveland Clinic, Cleveland, OH

Background: Fecal calprotectin is a valuable noninvasive marker for diagnosis and management of inflammatory bowel disease (IBD). The aim of our study is to evaluate the performance of two commercially-available and FDA-approved fecal calprotectin assays. Method: The Quanta Lite Calprotectin ELISA and Calprotectin Extended Range assay were obtained from Inova Diagnostics (San Diego, CA). Both assays were performed per manufacturer’s instructions. Analytical performance was evaluat-
ed following Clinical and Laboratory Standards Institute guidelines. Residual patient stool samples for calprotectin tests were used in the evaluation. Discordant results were assessed based on medical record review. Results: As shown in the table, the Calprotectin ELISA has better precision than the Extended Range assay. However, the Extended range assay showed much wider analytical measurement range (AMR), eliminating the need for additional dilution of samples. The measured calprotectin levels were categorized into normal (<50µg/g) and abnormal (>50µg/g). The agreement with a reference lab method using the PhiCal assay was 97.6% and 91.0% for Calprotectin ELISA and Extended Range assay respectively, and 94.1% between the Calprotectin ELISA and Extended Range assay. Although both methods correlated well with the reference lab method in quantitation, the slope and intercept of the Extended Range assay differed extensively from the other two assays. A large positive bias (about 50%) was observed between the calprotectin levels generated from the Extended Range assay and the reference lab method. Conclusion: Both assays showed acceptable agreement in identification of normal verses abnormal. However, calprotectin results generated from different assays are not comparable because of the large quantitative differences.

<table>
<thead>
<tr>
<th></th>
<th>Quanta Lite ELISA</th>
<th>Extended Range Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision Conc.</td>
<td>Total CV</td>
<td>Concentration Total CV</td>
</tr>
<tr>
<td>Control-L</td>
<td>22.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Control-H</td>
<td>141.3</td>
<td>2.7</td>
</tr>
<tr>
<td>Patient</td>
<td>326.8</td>
<td>9.0</td>
</tr>
<tr>
<td>AMR</td>
<td>15.6-220 µg/g</td>
<td>27.1-3000 µg/g</td>
</tr>
</tbody>
</table>

**Method comparison with a reference lab method using PhiCal assay**

- **X. Reference lab method.**
- **Y. Calprotectin ELISA.**
- **R**
  - 0.9485
  - 0.8992
- **Bias (%)**
  - 21.3 (68.8%)
  - 95.3 (46.1%)
- **Slope (95% conf.)**
  - 1.1 (1.0 to 1.2)
  - 1.6 (1.3 to 1.8)
- **Intercept (95%conf.)**
  - -10.0 (-68.8 to 48.8)
  - 7.1 (-51.1 to 65.2)

**A-359**

**Impact of obstructive sleep apnea on innate immune response and viral RNA quantitation in influenza virus infection: from host gene expression to primary cellular model**

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Background: Influenza virus infections can lead to acute respiratory distress syndrome and death in humans. Old age, male sex, medical comorbidities, and obesity are risk factors for the development of severe disease. These characteristics are also associated with obstructive sleep apnea (OSA). OSA may further compromise host immune response. However, the impact of OSA in influenza infection has not been reported. This study included two parts: 1) comparison of host gene expression of the upper respiratory tract (URT), with special regard to immune response, in patients with different severity of OSA, and 2) assessment of the effects of OSA on cytokine levels and influenza RNA quantity in primary cultures of human URT epithelial cells after H7N9 infection.

Methods: Part 1: Genomic RNA extracted from the fresh tissue of the uvula from 10 middle-aged patients without evident virus infection, 5 severe OSA (apnea-hypopnea index [AHI] ≥ 30) patients and 5 sex- and body weight-matched patients with mild OSA (AHI < 15), was used in RNA-Seq analysis. Differentially expressed genes (DEGs) and the absolute value of Log.Ratio ≥ 1 were considered for annotation analysis of gene ontology. Pathway analysis was performed in MetaCore™ to derive functional annotations. Part 2: Primary epithelial cells of the URT harvested from another 17 middle-aged patients undergoing airway surgery (10 severe OSA patients and 7 controls without evident OSA) were experimentally infected with H7N9 for 72 h. After virus infection, the culture media were collected for cytokine detection using the Bio-Plex Pro™ Human Cytokine 27-plex panel and viral RNA quantitation using real-time reverse transcription polymerase chain reaction at 24 h and 72 h.

Results: Part 1: There were 13 down-regulated and 45 up-regulated DEGs between severe OSA group and mild OSA group. Of them, 18 DEGs involved in immune system process. Furthermore, 86 significantly enriched pathways of immune response category including stress-induced antiviral cell response (ratio 54/57, P = 1.3e-13) and innate immune response to RNA viral infection (ratio 23/28, P = 4.0e-4) were identified. Part 2: At 24 h post infection, levels of IL-4, IL-6, IL-10, IFN-γ, MCP-1, and VEGF in patients with severe OSA were significantly higher than those of controls (all P < 0.05). The impact of OSA on cytokine levels was not evident at 72 h. Furthermore, the viral RNA quantities in primary epithelial cells from severe OSA patients and controls did not differ significantly at 24 h and 72 h, respectively.

Conclusion: Our preliminary findings demonstrate for the first time a clear distinction of host gene expression in regard to antiviral cell response and innate immune response to RNA viral infection between severe OSA patients and mild OSA patients. Although H7N9 infection of primary epithelial cells harvested from severe OSA patients can trigger rapid increases of several cytokines; however, these immune responses do not suppress viral replication. Our results suggest that OSA can potentially impact on innate immune response in influenza virus infections.

**A-360**

**Bronchoalveolar lavage findings in the diagnosis of patients with interstitial lung diseases**

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Background: Interstitial lung diseases (ILD) are a group of diseases that are the main pathological changes affecting the alveolar structures. The diagnosis of the patients is based on clinical symptoms, pulmonary function tests and radiological studies. When the diagnosis is unclear, invasive tests like bronchoalveolar lavage and pulmonary biopsy are used. The study of the bronchoalveolar lavage fluid in some interstitial lung diseases can reveal typical patterns to each disease that can support the diagnosis. The objective of this study was to perform a descriptive analysis of the cytologic study (lymphocytes, neutrophils, histiocytes and eosinophils) and the lymphocyte subpopulations in bronchoalveolar lavage fluid from patients with interstitial lung disease.

Methods: Retrospective study of the bronchoalveolar lavage fluid of 58 patients with ILD: sarcoidosis (SAR) (n=10), idiopathic pulmonary fibrosis (IPF) (n=12), nonspecific interstitial pneumonia (NSIP) (n=20), cryptogenic organizing pneumonia (COP) (n=7), and extrinsic allergic alveolitis (EAA) (n=9). The bronchoalveolar lavage fluid was analyzed to determine the distribution of cell populations and the lymphocyte subpopulations in bronchoalveolar lavage fluid from patients with interstitial lung disease.

Conclusions: The distribution of cell populations in bronchoalveolar lavage classified the interstitial lung diseases in three groups. Isolated lymphocytic alveolitis was found in SAR and isolated neutrophilic alveolitis was found in COP and IPF. Mixed alveolitis was the most common pattern in EAA and NSIP. The CD4:CD8 ratio was the most useful parameter in our study. The ratio was high in SAR (median, 5.80) and it was inverted in EAA (median, 0.19). It was low in the other interstitial lung diseases. The bronchoalveolar lavage fluid classification in interstitial lung diseases can reveal typical patterns to each disease that can support the diagnosis of the patient.
Evaluation of a Novel Multi-Analyte Assay for the Detection of Autoantibodies in the Diagnosis of Antihipophilid Syndrome (APS)


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Background: Antibodies to antiphospholipids (aPL) and associated proteins are a hallmark in the diagnosis of antiphospholipid syndrome (APS). Recently, a novel fully automated paramagnetic particle based multianalyte system (PMAS) has been developed which allows for the detection of autoantibodies to cardiolipin (aCL), beta 2 glycoproteinI (aβ2GPI), and to the phosphatidylserine/prothrombin (aPS/PT) complex. This study aimed to analyze the clinical performance of the novel system and compare to reference methods using clinically characterized samples. Methods: A total of 279 samples were collected from APS patients and various other disease controls (n=228) and were tested for aCL and aβ2GPI. Out of these samples, 104 from APS patients and 36 from disease controls were tested for aPS/PT. Antibodies were coupled to paramagnetic particles and tested using PMAS (research use only, Inova Diagnostics, San Diego, CA). A reduced number of samples in the patient population were also tested by reference methods, for comparison studies (QUANTA Flash CIA and QUANTA Lite ELISAs, Inova Diagnostics, San Diego, CA). Clinical sensitivity and specificity was calculated for all methods and comparative analysis was performed on the predicate device.

Results: The clinical performance for the novel aCL and aβ2GPI PMAS assays are outlined in the table below. Interestingly, for the first time we show good discrimination between APS and controls using the aPS/PT IgA isotype. Among the APS patients tested for all markers (aCL, aβ2GPI, aPS/PT), 50.0% of patients were aPS/PT positive only (negative for aCL and aβ2GPI). The correlation between platforms was good for aCL, aβ2GPI, and aPS/PT assays for all isotopes. The total agreement between aPS/PT assays is outlined in the table below.

Conclusion: Our data show excellent analytical and clinical performance of the PMAS as an aid in the diagnosis of APS. The addition of aPS/PT in the new system holds promise in the improvement for diagnosis and patient stratification.
(44.23-66.45) mg/L vs. 34.07 (26.31-52.89) mg/L, respectively (p<0.002). **Objective 3:** A moderate correlation was obtained between the (K-L) Index and the complement C4 (rho=0.495, p<0.0001) while no correlation was obtained with the complement C3 (rho=0.150, p=0.3) and anti-DNA antibodies (rho=0.199, p=0.3). **Objective 4:** The (K-L) Index was higher in patients with positive anti-DNA antibodies (anti-DNA=30 U/mL, n=23) vs. negative anti-DNA antibodies (anti-DNA=30 U/mL, n=25) [50.61 (43.89-63.90) mg/L vs. 27.28 (26.31-61.89) mg/L respectively (p=0.018)].

**Conclusions:** The (K-L) Index in patients with active SLE is significantly higher compared to patients with SLE in remission and healthy controls. In addition, the (K-L) Index allows to differentiate between patients with positive and negative anti-DNA. The results suggest that the (K-L) Index would reflect the activity of B cells in patients with SLE and it would be a potential biomarker of activity to be included in the follow-up of patients with SLE.

**A-364**

**Performance of A New Third Generation TRab Quantitative Assay**

on Fullyautomated Immunoassay Analyzer


**Background:** Hyperthyroidism in Graves’ disease (GD) is caused by autoantibodies to the TSH receptor (TSHR), and quantitative assay of the TSHR autoantibody (TRab) is widely used for diagnosis of GD and monitoring patients with GD. Fully-automated third generation TRab quantitative assays utilizing monoclonal antibody M22 have been launched, but most of the on-market TRab assays comprise a lyophilized TSHR-component which users need to reconstitute with liquid. We have developed a new third generation TRAB quantitative assay (ARCHITECT TRab) by using a liquid (ready-to-use) TSHR-component on the fully-automated chemiluminescent immunoassay analyzer.

**Objective:** To evaluate key performances of the newly developed prototype ARCHITECT TRab assay.

**Methods:** ARCHITECT TRab reagent lots under development were used. The assay needs 50ul of sample, 6-step calibrators from 0 to 50 IU/L and 29 minutes of reaction time for first result. Key performances were evaluated according to CLSI or similar protocols on precision (20 days; 2 reagent lots, 4 analyzers), limit of quantitation (LoQ) (2 reagent lots, 3 analyzers), linearity (1 reagent lot, 1 analyzer), method comparison (95 graves’ and 297 normal serum specimens; 3 reagent lots, 3 analyzers) with an on-market third generation TRab assay; Roche Elecsys Anti-TSHR and reagent on-board drift (2 reagent lots, 2 analyzers).

**Results:**
- **Total imprecision:** 8.1% to 11.4% (2.0 - 50 IU/L)
- **LoQ at 20% CV:** 0.6 to 0.9 IU/L
- **Linearity:** 0.9 to 40.7 IU/L with read-out value shifts within +/- 0.3 IU/L (2.0 IU/L) or +/- 15% (2.0 IU/L) from linear regression
- **Method comparison** with an on-market third generation TRab assay: Slope = 1.09 (passing bablok), r-value = 0.96
- **Reagent on-board drift** after one-time calibration: 7 to 14 days with read-out value shifts within +/- 0.3 IU/L (< 2.0 IU/L) or +/- 15% (2.0 IU/L)

**Conclusion:** The ARCHITECT TRab assay demonstrated good precision, sensitivity, linearity and reagent on-board stability. Method comparison data showed acceptable agreement with an on-market third generation TRab assay. The ARCHITECT TRab assay is expected to improve efficiency of TRab testing because of its improved usability by the read-to-use TSHR-component.

* Under development

**A-365**

**The diagnostic value of the AESKULISA PR3 sensitive & AESKULISA MPO in the EUVAS-cohort**

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**Objective:** Anti-neutrophil-cytoplasmic antibodies directed against proteinase-3 (PR3-ANCA) and myeloperoxidase (MPO-ANCA) are serological hallmarks of small vessel vasculitis, particularly granulomatosis with polyangiitis (GPA) and microscopic polyangiitis (MPA). In a recent multicentre European-Vasculitis-Study-Group (EUVAS) evaluation, the performance of IIF was compared to that of various antigen-specific immunoenasays. The aim was to evaluate the diagnostic accuracy of the third-generation antigen-specific immunosays PR3-ANCA (AESKULISA-PR3-sensitive) and MPO-ANCA (AESKULISA-MPO) to and compare these data with the results from the other assay (Oxgentec). **Methods:** 257 samples from the EUVAS cohort were tested for the presence of ANCA by PR3-ANCA ELISA (AESKULISA-PR3-sensitive) and MPO-ANCA ELISA (AESKULISA-MPO). Newly diagnosed GPA/MPA (n=66) patients and diseased controls (n=191) systemic lupus erythematosus (n=60), systemic sclerosis (n=10), rheumatoid arthritis (n=90), Scleroderma (n=11) and Sjögren’s syndrome (n=30) were analyzed.

**Results:** In AAV patients, ANCAs were detected with both methods in 56 cases; divergent results were obtained in only 1 patient sample. 191 patients with other rheumatic diseases were analyzed and only 13 vs 11 (AESKU/Oxgentec) were positive for ANCA (SLE, scleroderma, RA, RA/AV). This study shows that the PR3- and MPO-ANCA ELISAs are highly specific (93.2%/94.2%) and sensitive (85.9%/85.9%) in the detection of ANCA to identify AAV or conditions known to be associated ANCA. **Conclusions:** Our comparison of PR3- and MPO-ANCA ELISAS showed (i) a high diagnostic performance of these PR3- and MPO-ANCA ELISAS to discriminate AAV from disease controls. (ii) very good correlation between the other methods tested. In conclusion, these novel assays can be used as screening method for detection of ANCA-associated diseases.
### A-367

**Validation of Alzheimer’s Biomarkers: â-Amyloid 1-42 and Total Tau in CSF by Automated CleIA on Lumipulse G 1200 Platform**

**S. Narla, A. Dider, M. Hu, M. Florent. Covance Central Laboratories, Indianapolis, IN**

**Background:**
Guideline for Alzheimer’s disease diagnosis (AD) suggests using AD biomarkers for the pre-symptomatic and symptomatic phases. Cerebrospinal fluid (CSF) level of â-amyloid 1-42 (Aβ-42) and total Tau proteins have been increasingly included in the diagnostic process of Alzheimer’s disease. Aβ-42 is cleaved from amyloid precursor protein which ends up as aggregates in the brain, Aβ-42 plaque depositions are widely used to characterize AD. Analysis of Aβ-42 in CSF of AD patients shows significant reduction of Aβ-42 concentration. Tau is a neuronal protein which binds to microtubules in the neuronal axons. In healthy controls, levels of total Tau in CSF increase with age. Total tau levels are significantly enhanced in AD patients as compared with age-matched control subjects. Fujirebio (Fujirebio Inc., Japan) has developed fully automated chemiluminescence enzyme immunoassays (CLEIA) for analysis of Aβ-42 and total Tau in CSF. The purpose of this study is to evaluate the performance of these assays as per CLSI guidelines.

**Method:**
CSF Aβ-42 and total Tau are measured quantitatively by chemiluminescence enzyme immunoassay technology by a two-step immunoassay method on the Lumipulse G 1200 (Fujirebio Inc., Japan) using respective immunoreaction cartridges.

**Results:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experiment Design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aβ-42</td>
<td>3 levels, 20 replicates each, 1 day</td>
<td>Average % CV= 1.43</td>
</tr>
<tr>
<td></td>
<td>5 levels, 1 replicate per day, 10 days</td>
<td>Average % CV= 1.66</td>
</tr>
<tr>
<td>Total Tau</td>
<td>5 levels of CSF spiked with recombinant protein, 4 replicates each, 1 day</td>
<td>14 to 2069 pg/mL, slope = 0.977</td>
</tr>
<tr>
<td></td>
<td>3 levels, diluent spiked with recombinant protein, 3 replicates each per day, 3 days</td>
<td>14 pg/mL, CV = 10%</td>
</tr>
<tr>
<td></td>
<td>3 levels of QC, over 3 days at three time points (morning, afternoon and evening)</td>
<td>No significant change throughout the day</td>
</tr>
<tr>
<td></td>
<td>Not performed as samples not available</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Conclusion:**
The Access 3rd IS assay was successfully validated and demonstrated acceptable performance as per CLSI guidelines. Also, the assay is equivalent with both Access Hypersensitive (HS) TSH assay and with Siemens TSH 3 Ultra assay, especially at the lower end of the AMR.

### A-368

**Validation of new Access 3rd International Standard Thyroid Stimulating Hormone (TSH) Assay and its Correlation with Access Hypersensitive TSH and Siemens Ultra TSH assays.**

**S. Narla, C. Thomason, L. Pritchard, M. Florent. Covance Central Laboratories, Indianapolis, IN**

**Background:**
TSH is a glycoprotein consisting of two non-covalently bound subunits; an alpha and beta subunit. TSH released from the anterior pituitary is the principle regulator of thyroid function by stimulating the synthesis and release of thyroid hormones. Clinical use of measurement of TSH is for the assessment of thyroid status. The sensitivity of the TSH assay has increased substantially over last several decades leading to the current 3rd generation TSH assays. Current TSH methods across platforms claim traceability to the WHO 2nd international reference standard 80/558, and thus assays are expected to be equivalent. Upon depletion of the current WHO 2nd standard, WHO released the3rd international standard (3rd IS) 81/565. Beckman Coulter has released a new Access TSH assay that is standardized to 3rd IS (Access 3rd IS). The objective of this study is to validate the Access 3rd IS and determines its combinability with both Beckman Access Hypersensitive (HS) TSH assay and with Siemens TSH 3 Ultra assay.

**Method:**
Precision, accuracy, linearity, sensitivity and reference range of the Access 3rd IS are evaluated following CLSI guidelines. A minimum of 30 native samples covering the AMR of the TSH 3rd IS assay (0.01 – 50.0 mIU/mL) were analyzed in parallel on all three methods. Analytical Measuring Ranges (AMR) and Reference Ranges (RR) are, 0.01 – 50.0 m IU/L and 0.45 – 5.33 mIU/L, respectively, on the Access TSH 3rd IS; 0.02 – 100.0 mIU/mL and 0.34 – 5.60 mIU/mL, respectively, on Access the HS TSH; and 0.008 – 150 mIU/mL and 0.55 – 4.78 mIU/mL respectively, on Centaur TSH 3-ultra.

**Results:**

<table>
<thead>
<tr>
<th>Method</th>
<th>N</th>
<th>Range (mIU/mL)</th>
<th>Slope</th>
<th>Intercept</th>
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</tr>
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<tbody>
<tr>
<td>Access HS Vs. Access 3rd IS</td>
<td>32</td>
<td>0.540 – 33.970 (AMR)</td>
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<tr>
<td>Access HS Vs. Siemens 3 Ultra</td>
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<tr>
<td>Siemens 3 Ultra Vs. Access 3rd IS</td>
<td>32</td>
<td>0.533 – 35.782 (AMR)</td>
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<td>23</td>
<td>0.533 – 7.470 (RR)</td>
<td>1.045</td>
<td>-0.060</td>
<td>0.994</td>
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</table>

**Conclusion:**
The Access 3rd IS assay was successfully validated and demonstrated acceptable performance as per CLSI guidelines. Also, the assay is equivalent with both Access Hypersensitive (HS) TSH assay and with Siemens TSH 3 Ultra assay, especially at the lower end of the AMR.

### A-369

**New technology for improvement of sensitivity and specificity of IVD-Tests**

**U. Loos, S. Brith, R. Müller. KreLo GmbH Medical Diagnostics, Ulm, Germany**

**Background:**
Some test systems are lacking of high sensitivity and specificity which otherwise enable early and accurate diagnosis and treatment being beneficial for the course of the disease. This is known for Graves’ disease (GD), which is caused by thyroid stimulating immunoglobulins (TSI) directed against the thyrotropin receptor (TSHR). Current TSH or M22 displacement assays (TDAs) quantitatively indirectly all TSHR autoantibodies (Ab). We developed a direct epitope recognition assay (DERA) for sensitive detection of TSI in which the extracted capture hTSHR chimera is anchored on microtiter plates (sTRAb) and stimulatory epitope binding TSI are bridging to a soluble signalling chimeric extracellular domain (ECD) of the hTSHR. Here we show a Bridge assay in which a novel truncated capture hTSHR chimera is anchored on microtiter plates (sTRAb) and stimulatory epitope binding TSI are bridging to a soluble signalling chimeric extracellular domain (ECD) of the hTSHR. We show different signals from immobilised chimeric capture hTSHR, and thus we can bridge to a chimeric hTSHR ECD fused with secretory alkaline phosphatase or HRP. Applying chemiluminescence substrate sTRAb were quantified using a plate luminometer. The PMB assay was performed manually with a 12-tube magnet enabled ROC analysis of 190 samples (136 GD positive, 325 GD negative show a criterion for positivity > 0.54 IU/L with a sensitivity of 0.97 and a specificity of 0.99).

**Results:**

- The chimeric ECD of hTSHR-rLH/CG receptor shows a functionality/half life of 12/15 days at 4°C. ROC analysis of sTRAb DERA with 274 GD positive and 291 GD negative show a criterion for positivity > 0.54 IU/L with a sensitivity of 0.98 and a specificity of 0.99.

**Conclusion:**
The new technology for improvement of sensitivity and specificity of IVD-Tests is applicable in the clinical use of measurement of TSH for the assessment of thyroid status. Precision, accuracy, linearity, sensitivity and reference range of the Access 3rd IS are evaluated following CLSI guidelines. A minimum of 30 native samples covering the AMR of the TSH 3rd IS assay (0.01 – 50.0 mIU/mL) were analyzed in parallel on all three methods. Analytical Measuring Ranges (AMR) and Reference Ranges (RR) are, 0.01 – 50.0 m IU/L and 0.45 – 5.33 mIU/L, respectively, on the Access TSH 3rd IS; 0.02 – 100.0 mIU/mL and 0.34 – 5.60 mIU/mL, respectively, on Access the HS TSH; and 0.008 – 150 mIU/mL and 0.55 – 4.78 mIU/mL respectively, on Centaur TSH 3-ultra.

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of 94.1% and a specificity of 98.2%. In comparison the sensitivity/specificity/cut-off from the following tests: Roche Elecsys, cobas anti-TSHR 97.0%/99.0%/>1.75 IU/L; Thermo Brahms Kryptor: 96.3%/98.1%/1.80 IU/L; Immunex 2000/2001 TSI: 98.3%/99.7%/0.55 IU/L; xTRAh-DERA: 99.8%/99.1%/0.54 IU/L; PMB-Bridge assay 94.1%/98.2%/>1.50 IU/L. Further preliminary experiments with PMB performed on microtiter plates using the signaling hTSHR-ECR-HRP as detection receptor show a calibration curve reaching a clinical cut off of 0.2 IU/L. These results on microtiter plates are a basis for the development of automation for the PBM Bridge assay.

Conclusion:
For the first time an immobilized chimeric hTSHR ECD is shown to be excellently used on PBM in a Bridge assay which as a further innovation works with two secreted TSHR ECDs. Further improvement of technical statistics (sensitivity and specificity) is expected by transferring this prototype to fully automatic systems. Together with the good stability data automatization will improve sensitivity and specificity in comparison to other current IVD-assays by a novel technology. For the future we wish to apply this method for other autoimmune diseases such as diabetes type 1 in future cooperative studies. Also in diabetes type 1, early diagnosis and treatment will lead to less severe course of the disease.

A-370
Multiplexed, Isotype-specific Ultrasensitive Research-use Bridging Serology Assays for Detection of Autoimmune Reactivities
A. Mathew1, M. Wang2, S. Barbero1, K. Haynesworth1, W. E. Winter2, D. Pittman3, J. N. Wohlstadter2, 1Meso Scale Diagnostics, LLC., Rockville, MD, 2University of Florida College of Medicine, Gainesville, FL

Background: Autoimmune diseases affect over 50 million Americans and have rapidly rising incidence. The presence of multiple specific autoantibodies can often predict disease onset in at-risk individuals [e.g. type 1 diabetes (T1D), systemic lupus erythematosus, celiac disease], and assist in distinguishing disorders with similar clinical features (e.g. T1D versus type 2 diabetes). In an ongoing study to assess the feasibility of developing highly sensitive and specific multiplexed anti-germinating serology panels for detection of autoimmune biomarkers, the MSD® MULTI-ARRAY technology is being used to produce research-use assays for detection of T1D, other organ-specific autoimmune disease, and connective tissue disorder biomarkers. First generation multiplexed assay panels developed to detect T1D autoantibodies to glutamic acid decarboxylase (GAD), insulin 2 (IA2), and insulin (IAA), as well as markers relevant to celiac disease, autoimmune gastritis, and thyroid disease, were previously tested using assay proficiency evaluation samples from the Islet Autoantibody Standardization Program. In those studies, the MSD T1D-relevant assays performed comparably to existing assays, with the advantages of low sample requirements for multiplexed detection (less than 25 µL needed for detection of up to 10 biomarkers), high throughput, and no radioactivity [Mathew et al. (2016) Diabetes 65(Supplement 1):A431-A440, 1673P]. In the current phase of the project, next generation assays have been developed using MSD’s ultrasensitive assay format to enhance multiplexed detection of autoimmune disease-related reactivities with the additional capability to discern autoantibody isotypes.

Objective: The objective of the study was to compare performance of the first and second generation MSD bridging serology assay panels in a 96-well plate, high throughput format. The assays were assessed using commercially purchased T1D and presumably normal individual sera. The multiplexing capability enabled simultaneous quantitative measurement of T1D and comorbid disease markers. The second generation assay was formatted for detection of IgG reactivities, but can easily be formatted for detection of other antibody isotypes (IgA, IgM).

Results: Preliminary data show that assay sensitivities/specificities improve from 65%/100% to 91%/96% (IAA detection), 57%/100% to 70%/92% (GADA detection), and 35%/95% to 48%/96% (IA2A detection) when comparing the first and second generation assays, respectively. Furthermore, ultrasensitive assays were included for detection of pro-insulin autoantibodies (pro-IAA) and zinc transporter 8 (ZnT8) arrays, respectively. Two or more T1D reactivities were detected in eighteen of the twenty samples, with the good stability data presented so far.

Conclusion: The reported research was supported by the NIAID of the NIH under Award #1 U24 AI 118660. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.
Immunology

There was a 98.7% positive agreement and 100% negative agreement between the two methods, which gave a total agreement of 99%. Helios IFA pattern recognition ability scored a 95.8% agreement in comparison to the manual interpretation.

Conclusion: The correlation between the EIA and the Helios IFA methods for ANA screening and titer both showed excellent concordance. For those that were not in agreement during the initial screen but were further confirmed by ANA titer showed that the Helios IFA is by far a more sensitive method compared to EIA. Even though the Helios IFA titer pattern recognition is currently not FDA approved, it showed an excellent agreement to the manual gold standard method. Overall, the Helios platform is acceptable for performing ANA screen test and reflex titer confirmation in.

A-375
Atypical ANCA Testing with an Expanded ANCA Specificity Profile

J. B. Carter, S. Carter, M. J. Spalding, Lexington Medical Center, West Columbia, SC

BACKGROUND: Antibodies to Neutrophil Cytoplasmic Antigens (ANCAs) are associated with a variety of autoimmune diseases. C-ANCAs and P-ANCAs, when documented for PR3 or MPO specificity are important to the diagnosis and classification of autoimmune vasculitis. Standard testing involves screening for ANCAs by IFA [IIF], followed by a quantitative EIA method for anti-PR3 & MPO antibody levels. IFA ANCAs negative for both PR3 and MPO, if an ANA cross-reaction is excluded, have been termed an “Atypical ANCA”. We have used a 6 test Expanded ANCA Specificity EIA profile (Euroimmun) which includes testing for anti- MPO, PR3, Lactoferrin, Elastase, BPI, and Cathespin-G antibodies, resulting in detection of a variety of ANCAs specificities, and a significant decrease in the number of “Atypical ANCA” reports.

METHODOLOGY: All samples for ANCA testing are screened by IFA (INOVA) on both etha- nol and formalin-fixed slides, tested for anti-MPO & PR3 levels (INOVA EIA) and for ANA (Euroimmun HEP-2 IFA). Samples showing an ANCA-ANA positive for anti-PR3 or MPO specificity are reported with pattern and titer. Samples showing an IFA-ANCA, negative for both PR3 and MPO specificity, are tested by the Expanded ANCA EIA profile.

RESULTS: Results from a recent 24-month period show 998 (85%) of 1,170 samples to be negative, 172 (15%) positive for ANCA, 59 (34%) positives showed either PR3 or MPO ANCA, 52 (36% of positives) were termed “Atypical ANCA” as an antigen specificity was not evident. Use of the Expanded test ANCA profile showed 51 cases (30% of IFA-positive ANCAs) to have specificity for one or more of the additional antigens in the expanded profile. These results were sometimes crucial to establishing a specific diagnosis, and served to clarify an otherwise “Atypical ANCA”. BPI/ANCAs specificity was an isolated finding in 24 cases, each showing a C-ANCA pattern, and was often associated with severe infection. Elastase-ANCA [4 cases] occasionally correlated with Cocaine use, and Lactoferrin-ANCA [8 cases] occasionally with inflammatory bowel disease. Cathespin-G ANCA was noted in three (3) cases. Multiple ANCA specificities were noted in twelve (12) cases, eleven of which included at least one of the “new” ANCA specificities.

DISCUSSION: Fluorescent microscopic [IFA/IIF] detection of ANCAs is a global method showing visual evidence of patient antibody to a wide spectrum of neutrophil cytoplasmic and nuclear antigens, comparable to IFA detection of auto- antibodies in HEP-2 cells. Diagnostically valuable information is gained by testing for antibody specificity in both situations—ENA profile follow-up to a positive ANA, and an expanded antigen-specific profile following a positive ANCA result. Diagnostic information is limited when limiting ANCA follow-up testing to only MPO & PR3 specificities, and terming all other ANCAs as “Atypi- cal”. ANCA antigen-specificity proved more significant than ANCA IFA patterns.

CONCLUSION: Routine use of an Expanded ANCA Profile will further clarify and expand diagnostic utility of ANCA testing—similar to follow-up testing of an IFA- positive antinuclear antibody (ANA) with an expanded ENA profile.

A-376
Sensitive and multiplex detection of anti-islet cell autoantibodies for type 1 diabetes (T1D) diagnostics and risk assessments

J. Tsai1, P. Robinson2, S. Selleft1. ‘Stanford University, Palo Alto, CA, ‘En- able Biosciences, San Francisco, CA

Background: Clinical detection of anti-islet cell autoantibodies (autoAb) for type 1 diabetes (T1D) largely relies on radioimmunoassays (RIA). However, use of hazardous radioactive substance has limited its use to very few centralized clinical laboratories due to complicated waste disposal and regulatory processes. We expect a T1D auto/Ab assay that do not rely on radioactive materials could efficiently aid in decentralizing T1D testing and make the tests more affordable for wide arrays of patients in
Validation of a quantitative method for fecal zonulin

C. Cruzan, K. Urek, E. Roth, D. Quig, J. A. Maggiore. Doctor’s Data, Inc., Saint Charles, IL

Background: Zonulin is a human protein that appears to be the primary regulator of tight junctions between epithelial cells, including the epithelia of the intestinal tract. Zonulin binds to specific receptors on the surface of intestinal epithelia and triggers a cascade of biochemical events which induces tight junction disassembly and a subsequent increase in paracellular permeability of the intestinal epithelia. This increased permeability allows the influx of macromolecules from the intestinal lumen which invoke immune responses. Our goal was to validate a method for the reliable measurement of zonulin in feces. Increased levels of serum zonulin antigen are found in several autoimmune disorders, including celiac disease, insulin-dependent diabetes, multiple sclerosis, and rheumatoid arthritis. Fecal levels of zonulin have not been as extensively studied, but increased fecal zonulin levels have been reported in patients with metabolic syndrome.

Methods: The ImmunoDiagnostics Zonulin assay is based on a competitive enzyme linked immunosorbent assay (ELISA). A biotinylated zonulin tracer is added to samples, standards, and controls, which are subsequently transferred to a microtiter plate coated with polyclonal anti-zonulin antibodies. During incubation, the free zonulin in the sample competes with the biotinylated zonulin tracer for the polyclonal antibody immobilized in the microwells. Unbound components are removed by washing, and peroxidase-labeled streptavidin binds to the biotinylated tracer. After a second wash step, the substrate trimethylbenzidine is added to evoke a colorimetric response, and an acid solution stops the reaction. The optical density of standards, controls, and patient samples read at 450 nm is inversely proportional to the zonulin concentration. Analytical precision, linearity, recovery, limit of detection, and accuracy of this method were assessed, and sample stability was determined in freshly-collected fecal samples. Fecal samples provided by ambulatory adult volunteers (n=47) with no reported gastrointestinal maladies or autoimmunity disorders were used to determine a reference interval using EP Evaluator (Build 11.3).

Results: The intra-assay and total imprecision coefficients of variation (CV) of zonulin in fecal samples were determined as 5.7% and 13.0% at 35.0 ng/mL, and 6.7% and 15.4% at 60.9 ng/mL. Standard dilution of a fecal sample with increased zonulin demonstrated linearity (n=10) as 13.0-260.9 ng/mL, with recovery of 95.4% – 106.2%. The limit of detection was determined as 5.25 ng/mL. Adequate stability of zonulin in fecal samples was demonstrated for 11 days stored at 2.8°C or -20°C. Non-parametric statistical assessment of the data showed that the upper limit of the reference interval was 89.0 ng/mL (90% confidence interval: 80.1 – 97.9 ng/mL).

Conclusion: This ELISA method for fecal zonulin has been validated to be analyticaly precise and accurate, while providing a robust analytical range. Sample suitability has been established for the expedited temperature-controlled transportation of fecal samples from remote locations to a central laboratory for analysis. Further research is warranted to determine if a single measurement of fecal zonulin is associated with abnormal results for the established lactulose-mannitol test for paracellular permeability.


A-378

Evaluation of the HELIOS automated immunofluorescence processor/reader for ANCA and anti-DNA testing

T. Alexander, P. Agner. Summa Health, Akron, OH

BACKGROUND

Anti-neutrophil cytoplasmatic antibody (ANCA) testing and anti-double stranded DNA antibody (anti-DNA) testing are often performed by immunofluorescence assays (IFA). The IFA procedure is labor intensive and requires subjective interpretations by trained technologists. Automated slide processors and separate, automated image analysis microscopes have been developed to address these IFA shortcomings. These dual platform systems require operator intervention to transfer slides from the processor to the reader. The AESSKU HELIOS combines the processing and computer assisted image acquisition and analysis onto a single platform. Slides do not have to be transferred to a separate image acquiring microscope after processing, as both processes occur on the same instrument. Last year at this meeting we presented our evaluation of the HELIOS for anti nuclear antibody testing. In this study, we compared the HELIOS to manual IFA for ANCA and anti-DNA testing in a large community teaching hospital.

METHODS

For both ANCA and anti-DNA testing, we compared three different processing/reading methods. Method A- Automated Slide processing and automated interpretation on the HELIOS. Method B- Automated slide processing on the HELIOS; manual reading of the image on the computer by 2 independent, experienced readers. Method C- Manual slide processing and microscopic evaluation by the readers.

For the ANCA study, we analyzed 135 specimens from patients with autoimmune associated vasculitis, 120 from patients with documented ANCA and 375 from patients with other autoimmune and infectious diseases. All 630 specimens were tested at a 1:20 dilution on ethanol and formalin fixed neutrophils by the three different methods. For the anti-DNA study, we analyzed 297 specimens from patients with SLE and 479 from patients with other autoimmune and infectious diseases. All 776 specimens were tested on Crotidia laurensaeae slides at a 1:10 dilution using the three different methods.

RESULTS

ANCA- On ethanol fixed slides overall positive and negative agreement of all method comparisons and both readers ranged from 89.4-96.8%. Pattern agreements for all comparisons and readers ranged from 77.8-89.2% with the highest agreements between methods B and C; both methods of which relied on manual interpretation. On formalin fixed slides, overall positive and negative agreement between all methods and both readers ranged from 77.6-95.9%. Pattern agreements for all comparisons and readers ranged from 69.7-90.8% with the highest agreements between methods A and B. Anti-DNA- Overall agreements between all methods and both readers ranged from 80.3-97.5%. Positive agreements for all comparisons ranged from 44.8- 91% and negative agreements were 83.9-98.3%. The highest agreements were between methods B and C; both methods of which relied on manual interpretation.

CONCLUSION

The HELIOS automated slide processor/reader provides positive and negative qualitative results similar to manual processing and reading for ANCA and anti-DNA testing. No subjective difference was found between slides prepared manually and slides prepared on the automated HELIOS platform for either assay. Review of the HELIOS ANCA pattern interpretations by a trained reader will ensure that appropriate patient results are reported.

A-379

Development of functional assays for complement components C1q, C2 and C5.

C. E. Tange, A. R. Parker, S. J. Harding. The Binding Site, Birmingham, United Kingdom

Complement proteins form part of the innate immune system. Deficiencies in the complement system can manifest as a failure to mount an effective immune response or in autoimmunie conditions. Protein titer measurements in complement deficient patients can be misleading and functional tests may provide additional information. CH50 is a commonly used test to determine activity of the classical complement pathway. Abnormal results can indicate a problem with the production or consumption of one or more of the proteins in the classical complement pathway. Whilst useful, the measurement of CH50 does not help in identifying the specific deficiency. Here we describe simple adaptations to the CH50 Optitite® assay that can be performed on the Optitite analyser (The Binding Site Group Ltd., Birmingham, UK) to determine specific complement deficiencies. Mixing patient sera with sera depleted of a single known complement protein can de-
termine whether that functional protein is present or absent in the patient sample. Commercially available C2-depleted serum and sera from 2 healthy individuals were used to demonstrate the principle of the method. Undetectable CH50 activity was reported in the C2-depleted serum and CH50 results were 65.0 and 61.0 U/mL in healthy serum samples (normal CH50 reference range in serum: 41.68-95.06 U/mL). Mixing C2-depleted serum with sera from the healthy individuals (ratio 1:1) rescued CH50 activity (42.8 and 46.6 U/mL) indicating the presence of functional C2 in the healthy sera. Within- and between-sample variability was assessed for C1q, C2, C3 and C5-depleted serum. Within-sample variability was assessed by measuring CH50 activity in 10 replicates of a single premixed sample and between-sample variability was assessed by measuring CH50 activity in 10 individual healthy serum samples mixed 1:1 with complement-depleted serum. The within-sample coefficients of variation (CVs) ranged from 0.41% - 2.53% and between-sample CVs ranged from 7.29% - 11.68%. To validate the utility of this method in patient sera, CH50 activity was used to confirm a C2 deficiency in two patients. An undetectable C2 concentration (measured using Human Complement C2 SPAPLUS® assay, The Binding Site Group Ltd., Birmingham, UK) was reported in patient 1, who had a confirmed homozygous deficiency. Serum from patient 1 was combined with C2-depleted serum (ratio 1:1) and C5-depleted serum (ratio 1:1); CH50 assay results were undetectable and 27.9 U/mL, respectively. Thus the adapted method detected the absence of functional C2 and presence of functional C5 in the patient sample. A C2 concentration of 10.7 mg/L was reported for patient 2 (normal reference range: 18.7-44.0 mg/L). Consistent with this, when patient 2 serum was mixed with C2-depleted serum (ratio 1:1), CH50 activity was still below the normal reference interval (18.7 U/mL). In conclusion, a simple adaptation to the Optitite CH50 assay, mixing patient samples with commercially available complement-depleted serum, provides a simple method to detect absent or defective complement proteins.

**A-380**

Antibodies against neo-epitopes of microbial and human transglutaminases

T. Matthias, D. Agardh, P. Jeremias, S. Neidöhfer, A. Lerner, 1AESKU, KIPP Institute, Wendelsheim, Germany, 2Diabetes and celiac disease unit, Malmo, Sweden

Objectives and study: Microbial transglutaminase (mTg) and human tissue Tg (Ttg) form complexes with gliadin peptides, thus posttranslating and modifying gliadin to present neo-epitopes. The aims were to test the diagnostic performance of antibodies against both non-complexed and complexed forms of both transglutaminases in children with celiac disease, compared with disease controls and to correlate antibodies’ levels to the degree of intestinal atrophy. Methods: Serum samples, at day of intestinal biopsy, were collected from 350 children with celiac disease (mean age 7.4 years) and 215 disease controls (mean age 10.2 years) and tested using the following ELISAs detecting IgA, IgG or both IgA+IgG combined with celiac disease (mean age 7.4 years) and 215 disease controls (mean age 10.2 years)

Results: In patients, the tTg neo Check had the highest sensitivity and tTg IgA the highest specificity. Comparing the different correlations between antibodies’ iso-types, the tTg Check (r = 0.7889, p < 0.0001) and tTg neo Check (r = 0.5544, p < 0.0001) as well as tTg IgA and tTg neo IgA (r = 0.7571 and r = 0.7279, p < 0.0001) respectively were the best indicators of intestinal damage in children. Conclusion: It is suggested that the combination of tTg neo IgA/IgG antibodies should be recommended as a first line screening test for CD in children. The tTg and tTg neo assays show similar diagnostic performance and are recommended as good screening tests for CD in children. mTg neo IgG presents a new serological biomarker for celiac disease.

**A-382**

IgG, IgA and IgM responses to pneumococcal polysaccharide vaccination (Pneumovax®) in a normal healthy adult population

A. Parker1, S. Harding1, R. Abraham2, 1The Binding Site Group Ltd, Birmingham, United Kingdom, 2Mayo Clinic, Rochester, MN

Background: Assessment of the response to pneumococcal polysaccharide vaccin- nation (Pneumovax®, PPV) may help identify antibody deficient patients who are most at risk of infection. A complete response to PPV would include production of antigen-specific IgG, IgA and IgM antibodies, but currently immunological investiga- tion only requires measurement of the IgG isotype. In this study, we determined the concentration ranges of pneumococcal IgG, IgG2, IgA and IgM in response to Pneu-

**A-383**

Development of a 3 step screening procedure for the measurement of pneumococcal antibodies

A. Parker, M. Reynolds, S. Harding, M. Synder1, 1The Binding Site Group Ltd, Birmingham, United Kingdom, 2Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

Background: Currently there are two different methods by which pneumococcal sero-type antibodies may be quantified: measurement of the summation of all serotype specif-
ic pneumococcal antibodies or measurement of the antibodies individually, usually in a multiplex format. Here, an algorithm for the measurement of pneumococcal antibodies that screens using summation and reflexes to individual serotype analysis is presented. Methods: Serum samples were obtained from 1510 individuals referred to the Mayo Clinic. Serotype antibody concentrations for all 23 serotypes were available from 1264 individuals (M:F ratio 499:765; median age 38 years, range 2-105). For validation of the algorithm, normal healthy volunteers (n=89; M: 44.5; median age 44 years, range 20-66) and CVID patients (n=36; M: 14.22; median age 39 years, range 14-74) post Pneumovax® 23 vaccination were analysed. Total pneumococcal IgG antibodies was measured using a commercially available ELISA kit (VaccYze™ Pneumococcal cap-
sular polysaccharide (PCP) IgG ELISAs. The Binding Site Group Limited, UK, mea-
sured 3.3-270 mg/L and Pneumococcal antibody serotype analysis was performed for 23 antibodies using a laboratory defined test based on Luminox technology.

Results: Cut-offs of 9.7 mg/L and 270 mg/L in the PCP IgG ELISA were identified based on high confidence that either an antibody deficiency (<9.7 mg/L) or healthy response (>270 mg/L) would be identified by both methods. The agreement between both methods at each cut off was 99%. Samples with a concentration between 9.7 mg/L and 270 mg/L would be candidates for reflexing to pneumococcal serotyping. To further “screen” these antibodies using the simple PCP IgG ELISA, two further indeterminate cut-offs at 40 and 180 mg/L were established. At these cut-offs, agreement between the total and serotyping assays was decreased (82% and 95% respectively). However, given the still significant agreement, our algorithm proposes that clinician discussion/clinical information should form part of the decision making process for reflexing to serotyping. Samples with a pneumococcal antibody concentration between the indeterminate ranges (e.g. 40-180 mg/L) would be automatic candidates for pneumococcal serotyping analysis which would account for 44% of total samples (554/1264). In order to validate use of the total PCP assay as a screening test prior to serotype-specific testing, the proposed cut-offs were applied to a cohort of healthy individuals (n=89) and CVID patients (n=36). Of the 12 individuals with total PCP results <9.8 mg/L, 11 (91.6%) carried a diagnosis of CVID. In contrast, of the 51 samples with total PCP results >270 mg/L, all were identified as healthy controls. Within the lower indeterminate range (9.7-40 mg/L), 11 out of the 12 (91.6%) were from patients with CVID, in the higher indeterminate range (180-270 mg/L), 11 out of the 12 (91.6%) were from healthy individuals. Lastly, the range of 40-180 mg/L showed the

**Immunology**

<table>
<thead>
<tr>
<th>Medium pre- and post- PPV vaccination concentrations (and 95% CI) for PPV IgG, IgG2, IgA and IgM</th>
<th>Vaccination pre/post</th>
<th>Pre</th>
<th>4.6 weeks post</th>
<th>6 months post</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (mg/L)</td>
<td>43 (11-186)</td>
<td>375 (77-1238)</td>
<td>151 (31-1073)</td>
<td></td>
</tr>
<tr>
<td>IgG2 (mg/L)</td>
<td>18 (4-120)</td>
<td>141 (25-573)</td>
<td>59 (10-473)</td>
<td></td>
</tr>
<tr>
<td>IgA (U/mL)</td>
<td>22 (6-182)</td>
<td>369 (78-1802)</td>
<td>85 (19-279)</td>
<td></td>
</tr>
<tr>
<td>IgM (U/mL)</td>
<td>53 (16-168)</td>
<td>315 (60-1133)</td>
<td>54 (17-128)</td>
<td></td>
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</tbody>
</table>
Vitamin D and disease activity in patients with rheumatoid arthritis


Background: Vitamin D has immunomodulatory effects in a wide variety of chronic diseases (autoimmune, cardiovascular, oncological, ...). Its deficiency has been related to the presence and activity of autoimmune diseases such as rheumatoid arthritis (RA). 25-hydroxyvitamin D or 25(OH)D has a half-life of 2-3 weeks, making it the best serological biomarker of vitamin D status in the body. The main objective of the study is to evaluate the relationship between 25(OH)D levels and the severity of the disease in patients with RA. The following objectives were established: 1) Compare the levels of 25(OH)D between healthy controls and patients with RA. 2) Compare the levels of 25(OH)D according to disease activity. 3) Evaluate the status of vitamin D in patients with RA. 4) Evaluate the correlation between 25(OH)D levels and the clinical parameters of the disease: number of painful joints (NAD), number of inflamed joints (NAI), DAS28 activity index and biomarkers (PCR, FR and anti-CCP).

Results: The study subjects had a mean age of 32±9.0 years (opiate dependents) and 51 years (45-57) and 78 patients with RA (18-men:60-women, age=57 years (49-65)). 29 patients in remission and 49 patients with active disease. The activity of the disease was evaluated with the use of the DAS28 index: remission (DAS28 <2.6) and active disease (DAS28 >2.6). Vitamin D status was defined as deficiency (25(OH)D<20 ng/mL), insufficient (25(OH)D between 20-30 ng/mL) and adequate (25(OH)D between 30-100 ng/mL). The levels of 25(OH)D, DAS28, NAI, NAD, PCR, FR and anti-CCP. The Mann-Whitney test was used for comparisons between 2 quantitative variables, the Chi-square test for comparisons between qualitative variables based on contingency tables and the Spearman correlation analysis to study the correlation between variables. A p<0.05 was considered statistically significant.

Conclusions: The levels of 25(OH)D were lower in patients with RA than in healthy controls. Vitamin D deficiency was associated with the clinical activity of the disease. The quantification of serum 25 (OH) D levels and, consequently, vitamin D supplementation should be considered in the management of patients with RA.
Comparison of a conventional and a nucleosome linker-based anti-dsDNA ELISA

J. Dunphy1, S. Kramp2, N. Johannsen3, P. Hamann1, O. Sendscheid1, C. Dähnrich4, W. Schlumberger5, N. McHugh6. 1Department of Pharmacy and Pharmacology, University of Bath and Department of Haematology and Immunology, Royal United Hospitals Bath NHS Foundation Trust, Bath, United Kingdom, 2Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Lübeck, Germany, 3Department of Pharmacy and Pharmacology; University of Bath; Bath, Germany, 4EUROIMMUN US, Inc., Mountain Lakes, NJ, 5Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom

Background: Autoantibodies against double-stranded deoxyribonucleic acid (dsDNA) are found almost exclusively in systemic lupus erythematosus (SLE) and present a well-established criterion for diagnosis. Anti-dsDNA autoantibodies can be detected by different methods. In a conventional ELISA the target antigen dsDNA is linked to the solid phase by negatively charged molecules. As these are prone to cause unspecific reactions, the Euroimmun Anti-dsDNA-NCX ELISA uses purified mono-nucleosomes instead. This study was designed to compare the diagnostic performance of a conventional ELISA with the NCX-ELISA.

Methods: Autoantibodies of class IgG against dsDNA were measured in serum samples from 204 SLE patients (including patients with inactive SLE under treatment) and 128 non-SLE patients (including rheumatoid or psoriatic arthritis, osteoarthritis, (fibro)myalgia, Raynaud’s phenomenon, cutaneous sclerosis and vasculitis) using a conventional anti-dsDNA ELISA (QUANTA Lite®, Inova, USA) and the nucleosome linker-based Anti-dsDNA-NCX ELISA (Euroimmun, Germany).

Results: The QUANTA Lite® dsDNA ELISA revealed a diagnostic sensitivity of 22.1% at a specificity of 84.4% and the anti-dsDNA-NCX ELISA a diagnostic sensitivity of 29.4% at a specificity of 93.0%. The specificity of the QUANTA Lite® dsDNA ELISA is set to equivalent 93.0%, ROC curve analysis demonstrates a sensitivity of only 15.2%.

Conclusion: The Anti-dsDNA-NCX ELISA, utilizing purified mono-nucleosomes as a linker for dsDNA, is superior in sensitivity and specificity than a conventional anti-dsDNA ELISA.

Preferential kappa selection in circulating human immunoglobulins: an interesting conundrum for immunoglobulin gene rearrangement

M. Hetrick1, O. Martinez2, M. Kohlhagen1, D. Murray1, S. Dasari1, 1Mayo Clinic, Rochester, MN, 2Winona State University, Winona, MN

Background: The circulating antibody population is commonly used as a surrogate marker for B-cell gene selection. Analysis of the antibody repertoire by mass spectrometry showed unexpected bimodal mass distributions in total human serum samples. Kappa (κ) light chain (LC) but not lambda (λ) LCs (Barnidge et al. 2015). The prevalences of autoantibodies against these antigens can then be determined in corresponding index serum. The strategy is currently being applied to further UFO-ANA.

Methods: 36 serum samples with pre-determined UFO-ANA against unknown nuclear autoantigens as defined by a pattern on Hep-2 in indirect immunofluorescence assay (IFA) were selected and presented a well-established criterion for diagnosis. Anti-dsDNA autoantibodies can be detected by different methods. In a conventional ELISA the target antigen dsDNA is linked to the solid phase by negatively charged molecules. As these are prone to cause unspecific reactions, the Euroimmun Anti-dsDNA-NCX ELISA uses purified mono-nucleosomes instead. This study was designed to compare the diagnostic performance of a conventional ELISA with the NCX-ELISA.

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Results: Of the 162 samples tested, the numbers of qualified spectra used for each of the calculations were as follows: 94 IgGκ, 162 IgGκ, 126 IgGκ, 53 IgGκ, 40 IgGκ, and 53 IgGκ. The average ratio of heavy-κ LC to normal κ LC ranged from 0.58 to 0.35. IgG1 had the highest proportion of heavy-κ LC (0.58) followed by IgG4 (0.540) then IgG3 (0.482), total IgG (0.479), and IgG4 (0.398). IgM contained a significantly greater heavy-κ/k ratio than IgG3 (p=0.0367), total IgG (p=0.00265), IgG4 (p=0.0001), and IgGκ (p=0.0001). Conclusions: A population of heavy-κ LCs, unique from the normal mass k LC distribution was paired to all isotypes investigated (IgA, IgG1, IgG3, IgG4, and IgM). A significantly higher amount of heavy-κ LC was found in IgM as compared to other isotopes except IgG1, suggesting that circulating human immunoglobulins have a deterministic mechanism to select one type of k LC based on Ig class and subclass. These findings present an interesting conundrum for current gene light chain selection theory.

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J. Dunphy1, S. Kramp2, N. Johannsen3, P. Hamann1, O. Sendscheid1, C. Dähnrich4, W. Schlumberger5, N. McHugh6. 1Department of Pharmacy and Pharmacology, University of Bath and Department of Haematology and Immunology, Royal United Hospitals Bath NHS Foundation Trust, Bath, United Kingdom, 2Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Lübeck, Germany, 3Department of Pharmacy and Pharmacology; University of Bath; Bath, Germany, 4EUROIMMUN US, Inc., Mountain Lakes, NJ, 5Department of Pharmacy and Pharmacology, University of Bath, Bath, United Kingdom

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Quantitating the M-protein: A comparison between the perpendicular drop and the tangent skimming methods on Agarose Gel (AGE) and Capillary Zone (CZE) Electrophoresis

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BACKGROUND: Serum protein electrophoresis (SPEP) is a staple test for detecting and measuring the M-protein. Different techniques exist for quantitating M-proteins: perpendicular drop (PD) or tangent skimming (TS). It’s been reported that TS would be more accurate at lower concentrations (<1g/dL) and favored for quantitation. This study compared PD and TS methods in different institutions using CZE or AGE on gamma-migrating M-proteins.

METHODS: Residual waste serum with physician-ordered SPEP was used to create hypogamma, normal, and hypergamma background samples, then divided into 2 groups (n=6). Each pool was spiked with elotuzumab (Elo) or daratumumab (Dara) monoclonal antibodies (mAbs). MAb concentration in each pool ranged from 1-0.02g/dL. SPEP was performed in duplicate in different analytical runs (n=62). Mayo Clinic (MC) used a SPIFE SPE Vis agarose gel using a Helena SPIFE 3000 unit and Helena QuickScan 2000 densitometer, gating mAbs first with PD, then with TS. University of Michigan-Ann Arbor (UM), performed SPEP by CZE on the Sebia Capillaries 2, and mAb concentrations were determined using TS. A recovery within 80-120% from the expected concentration was considered acceptable.

RESULTS: Percent recovery was calculated for three sets of data gated by different methods (Table). Zero percent (0/66) of samples analyzed by AGE/TS fell within the acceptance criteria (80-120%). Thirty percent (20/66) of all samples analyzed by AGE/PD and 59% (39/66) of samples analyzed by CZE/TS met those criteria. Lowest measured concentrations with acceptable recovery were 0.02g/dL for CZE/TS and 0.2g/dL for AGE/PD. The hypogamma background affected the PD method more significantly than TS.

CONCLUSION: Our findings suggest that SPEP performed by CZE/TS showed improved accuracy compared to AGE/PD or AGE/TS at mAb concentrations <1g/dL, and that the system used for quantitation also plays a critical role in the accuracy of the reported result.

Discrepant CMV IgM Immunoassays: A Comparison between Bio-Rad RCM and TORCM with Poor Positive Agreement

A. Barbieri, R. Huang, H. Sun, X. Yi. Houston Methodist Hospital, Houston, TX

Background: Cytomegalovirus (CMV) infection in the general, immunocompetent, population is mostly asymptomatic. However, CMV infection is of great concern in patients with weakened immune systems; and therefore, determination of CMV infection status in immunocompromised patients is of utmost importance, including patients with HIV infection, patients receiving treatment for autoimmune conditions, infants with not yet fully developed immune systems, and organ transplant recipients, as well as an important component of pre-transplant testing. CMV tends to infect transplant organs and is thought to contribute to chronic rejection. CMV IgM immunoassays aid in establishment of primary CMV infection, as well as secondary infection, with a different strain or reactivation of a latent CMV strain.

Methods: In this study we evaluated two semi-quantitative CMV IgM immunoassays from Bio-Rad®: the latest generation TORCM and the previous generation RCM. Positive and negative quality controls were run in duplicates for 10 days to assess precision of the TORCM assay. A total of 40 patient serum samples were tested by the two different assays on the same instrument platform (Bioplex 2200, Bio-Rad). The discrepant results were compared to a third commercially available immunoassay (DiaSorin LIAISON XL) and were also correlated with available CMV PCR test data for each patient, if tested at the same time period.

Results: The new TORCM assay shown good precision with 100% agreement for both positive and negative control. Of the 40 tested samples, a total of 21 samples were tested negative by both TORCM and RCM, with 100% negative agreement between the two assays. Out of 18 samples tested positive by the RCM assay, 7 samples had discrepant results by TORCM assay, with only 61% positive agreement between the two assays. One remaining sample tested equivocal on RCM and positive on TORCM. Of the 7 discrepant results, 6 agreed between TORCM and DiaSorin. CMV PCR test data was available in 9 of the 18 positive samples by RCM assay. When compared to the available CMV PCR data, both assays showed a poor total agreement with the PCR results: RCM, 33%; TORCM, 44%.

Conclusion: This study demonstrates poor positive agreement between the two Bio-Rad CMV IgM immunoassays. These results warrant further test comparison with standard molecular data, and further demonstrate a concerning and inherent lack of positive agreement between two different CMV IgM immunoassays with significant potential impact in patient care.