Detection of biofilm forming bacteria in clinical isolates and their antimicrobial susceptibility pattern

P. Neopane¹, H. P. Nepal³, R. Gautam¹, S. Ansari¹, S. Shrestha¹, S. Thapa¹, R. Shrestha¹. ¹Chitwan Medical College, Bharatpur, Nepal, ²Department of Microbiology, Trinity School of Medicine, St. Vincent and the Grenadines, Saint Vincent and the Grenadines, ³Faculty of Medicine, Oita University, Oita, Japan, ⁴Faculty of Health Sciences, Hokkaido University, Sapporo, Japan

Background: Biofilm is a structured community of bacterial cells enclosed in a self-produced polymeric matrix adherent to a surface. About 60% human infections (either foreign body infections or native tissue infections) are biofilm related. The biofilms are of great medical importance as they are found to decrease antimicrobial susceptibility of the associated pathogens. Furthermore, the proximity of cells within a biofilm can facilitate a plasmodium exchange and hence enhance the spread of antimicrobial resistance. Due to the increasing rate of multidrug resistant organisms in Nepal, it is very essential to detect the occurrence of biofilm production, which could be the reason of multidrug treatment failure. Moreover, understanding biofilm production rates and antimicrobial resistance patterns could help us to formulate the antibiotic policy for effective treatment. Therefore, this study aimed to detect biofilm forming clinical isolates, determine their antimicrobial resistance with special reference to multidrug resistance (MDR), extended spectrum β-lactamase (ESBL) production and methicillin resistance and compare MDR, ESBL producers and Meticillin-resistant Staphylococcus aureus (MRSA) isolates among biofilm producers and non-producers.

Methods: A descriptive cross sectional study was carried out in 300 total clinical isolates, of which E.coli were 76, Klebsiella spp. 65, Pseudomonas aeruginosa 55, Acinetobacter spp. 54 and Staphylococcus aureus 50. Biofilm formation was detected by using two methods namely, tube adherence method and microtiter plate method. Antimicrobial susceptibility test was performed by modified Kirby Bauer disk diffusion method. Rate of MDR, ESBL production and occurrence of MRSA were determined among biofilm producers and non-producers.

Results: Biofilm formation was detected as 67.3% by microtitre plate culture method and 66.3% by tube adherence method. Considering TCP method as a gold standard, the sensitivity, specificity, positive and negative predictive values of TM in the present study were 84.2%, 70.4%, 85.4%, 68.3% respectively. Biofilm producers exhibited significantly higher rate of antimicrobial resistance than did biofilm non-producers. MDR and ESBL production among biofilm producers were significantly higher (68.8% and 51.8% respectively) than biofilm non-producers (40.8% and 17.8% respectively). Similarly, MRSA among biofilm producing Staphylococcus aureus were 44.4% whereas none of the biofilm non-producers were found to be MRSA.

Conclusion: In conclusion, two third of common isolates that are routinely encountered in our clinical laboratory are biofilm producers. Higher rate of antimicrobial resistance exhibited by biofilm producers than by biofilm non-producers in the present study emphasizes that biofilm is one of the important causes of treatment failure. Therefore, regular surveillance and early detection of biofilm forming isolates, determination of their antibiogram, and implementation of proper antimicrobial guidelines are crucial in reducing the burden of resistant infections in health care setting.

Development of alternative diagnostic methods for acute, chronic, and post-treatment phases of Schistosoma mansoni infection

V. S. Moraes¹, P. Z. Coelho¹, D. Harn¹, L. V. Siqueira¹, N. B. F. Almeida¹, W. C. Borges², R. F. Q. Fenrell², L. M. Shollenberger². ¹Rene Rachou Research Center - Oswaldo Cruz Foundation, Belo Horizonte, Brazil, ²University of Georgia, Athens, GA, ³University of Oporto Preto, Ouro Preto, Brazil

Background: Schistosomiasis is a serious global public health problem. The standard for diagnosis of infection is the Kato-Katz method, which has low sensitivity and therefore does not work well on patients with low-level infections, representing the majority of cases. Thus, the development of improved diagnostic tests is necessary for disease control. Adding tests such as ELISAs using soluble egg antigens (SEA), increases diagnostic accuracy in low burden endemic and non-endemic areas of Minas Gerais, Brazil. However, crude SEA antigens have low specificity and cross-react with other helminthes. Therefore, the goal of this work is to identify SEA proteins with high schistosomiasis specificity, as well as the sensitivity to differentiate between active (acute and chronic) and cured (post-treatment phase) infections, in order to develop point-of-care (POC) tests and improve traditional ELISAs. Methods: SEA was generated from livers of female Swiss Webster mice 45 days after infection with Schistosoma mansoni. Using a protocol approved by the Brazilian Ethical Committee, human serum was obtained in Minas Gerais from each group: healthy volunteers (negative controls); schistosome acute, chronic and post-treatment patients; and patients infected with other helminthes. Fifteen serum samples from each group were pooled and submitted to two-dimensional Western blot (2D-WB) using 60 μg of native SEA. 2D-WB were repeated using sodium metaperiodate (SMP) treated SEA, to denature sugar moieties. The immunoreactive spots were identified by mass spectrometry and analyzed by bioinformatics tools. Promising sequences will be cloned, proteins expressed, and monoclonal antibodies produced. Results: A majority of the 23 spots identified by serum from Schistosoma-infected patients were related to housekeeping proteins (heat shock, energetic metabolism, structural). Among these, 22 spots were identified by serum from patients infected with other helminthes, and 9 by negative control samples. One spot was uniquely recognized by sera from Schistosoma-infected patients and detection remained after sugar denaturation by SMP treatment, suggesting serum antibodies were binding to peptide epitopes. Conclusions: We identified putative new schistosome molecules, which will be produced as recombinant proteins. These proteins will be combined with monoclonal antibodies in the development of highly specific ELISAs and POC immunodiagnostic tests. These tests will be invaluable due to their high specificity and sensitivity as well as their ability to distinguish active and treated cases. Further, the fast, simple POC assay requires minimal equipment and will be an accurate screening tool for epidemiologic surveying in low resource regions.

Paraoxonase 1 (PON1) and Oxidative Status in Patients with Active Pulmonary Tuberculosis

A. Nagila, Gandaki Medical College and teaching Hospital, Pokhara, Nepal

Background: Paraoxonase 1 (PON1), a high density lipoprotein (HDL)-associated esterase/lactonase is a potent antioxidant and its activity is influenced by PON1 polymorphism. Mycobacterial infection induces oxidative stress which might promote tissue injury and inflammation. However, the PON1 activity in Tuberculosis infection remains poorly understood. Therefore, the aim of this study was to investigate the effect of Tuberculosis infection on oxidative status and PON1 activity and to explore the polymorphism of PON1, Q192R, L55M genes in Pulmonary Tuberculosis (PTB) patients.

Methods: A total of 108 (52 newly diagnosed active PTB and 46 healthy control) subjects were recruited from the Western Regional Tuberculosis Center, Pokhara, Nepal for this study with the mean age of 37.3±1.72 years. Anthropometric variables, Lipid profile, total protein, albumin, uric acid, glycosylated haemoglobin (HbA1c), C reactive protein (CRP), total peroxide, total antioxidant substance, oxidative stress status, PON1 arylesterase and paraoxonase activities were determined in control and PTB subjects. PON1, Q192R, L55M polymorphisms were also determined in both healthy controls and PTB patients

Results: Significant difference in BMI, SBP and DBP was observed between PTB and control subjects (p<0.001, p<0.01, total p<0.001, Western blot). TC and HDL were significantly lower in PTB subjects in comparison to healthy controls (p<0.05, p<0.001, p<0.01 and p<0.01, respectively). Although, HbA1c, TG and LDL levels were also found decreased, were not statistically significant. The level of CRP, uric acid and globulin were significantly increased PTB patients (p<0.01, p<0.01, p<0.001, respectively). Serum PON1 arylesterase and paraoxonase activity and total antioxidant substance were significantly lower in patients than control (p<0.001, p<0.001, total p<0.001) while p-total peroxide level and oxidative stress level were significantly higher (p<0.01, p<0.001, respectively). In PTB patients oxidative stress level was significantly correlated with PON1 paraoxonase activity (r= 0.390, p< 0.001). A statistically significant difference in PON1 L55M polymorphism was found between the PTB patients and the control group (p=0.05), however, there was no statistically significant difference in PON1 Q192R polymorphism between control and patients group (p=0.05).

Conclusion: Patients with active pulmonary tuberculosis are exposed to potent oxidative stress and decreased PON1 activity. These predisposing factors might play a role in inflammation and the pathogenesis of atherosclerosis in PTB.
An Evaluation of Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay at two European Trial Sites.

C. Chandler1, P. Contestable1, L. Holm Harristhoj2, T. Kjerstadius3. 1Ortho Clinical Diagnostics, Marlow, Buckinghamshire, United Kingdom. 2Ortho Clinical Diagnostics, Rochester, NY. 3Rigshospitalet, Copenhagen, Denmark. 4Central sjukhuset Karlstad, Karlstad, Sweden

Objective: To assess the clinical performance (sensitivity and specificity) of the VITROS Immunodiagnostic Products HIV Combo Assay* on VITROS Systems with Microwell capability, in routine use at two external testing laboratories in Europe. The assay is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection.

Methods: Antibody detection in the VITROS HIV Combo Assay* is achieved using recombinant transmembrane envelope proteins for HIV-1 group M and O and HIV-2. The p24 antigen detection is accomplished using monoclonal antibodies (MAbs) against HIV p24. Biotinylated antigen or MAbs are pre-bound to microwells coated with streptavidin. Sample is added to the coated wells in the first stage of the reaction and HIV analyte from the sample is captured by the biotinylated proteins. After washing, HRP conjugated envelope proteins and anti-p24 MAbs are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent.

Results: The specificity and sensitivity testing was performed at two external hospital laboratories, one in Denmark and one in Sweden. The Danish hospital laboratory used one assay lot on both a VITROS 5600 Integrated System or a VITROS ECI/ECIQ Immuno-diagnostic System. The Swedish laboratory used a different assay lot, run on a VITROS 3600 Immuno-diagnostic System. Assay specificity was estimated using 5077 blood donor samples and 607 samples from hospitalised patients. Assay sensitivity was evaluated by running 20 commercially available seroconversion panels and 34 fresh (less than 24 hours old) known HIV positive patient samples. All sample results were compared against results from a commercially available fourth generation HIV Combo assay.

Conclusion: The VITROS HIV Combo Assay* provides comparable sensitivity and specificity performance when compared with a commercially available 4th generation assay.

*In Development

A Performance of a Prototype T. cruzi Assay* on the VITROS® ECI/ECIQ and 3600 Immunodiagnstics Systems for the Detection of Chagas Disease

S. J. Danielson, T. Grupp, J. Ramer. Ortho Clinical Diagnostics, Rochester, NY

Background / Objective: Chagas Disease is caused by infection with the blood-borne protozoan parasite Trypanosoma cruzi (T. cruzi). The acute infection can resolve into a chronic, asymptomatic stage during which the infection can still be transmitted. This disease is endemic in many parts of Latin America. We have assessed the performance of a prototype T. cruzi Assay* on VITROS Immunodiagnostic Systems.

Method: Antibody detection in the VITROS® T. cruzi Assay* is achieved using antigen coated microwells which capture T. cruzi antibodies present in the sample. After addition of the HRP-labeled anti-human IgG and the VITROS® Signal Reagent, the bound HRP conjugate is measured by a chemiluminescent reaction. The signal is compared to a cutoff signal generated using a positive calibrator. All specificity and sensitivity testing was performed using one lot of reagent on a VITROS ECIQ Immunodiagnostic System with the exception of the dilutional, seroconversion, and performance panels which were tested on a VITROS ECIQ and a VITROS 3600 Immunodiagnostic System to compare performance across systems. Assay specificity was assessed using 1361 serum and plasma blood donor samples and 142 samples from individuals with unrelated medical conditions. Assay sensitivity was evaluated by testing 105 serological presumed positive samples from a diverse geographic endemic population, and 2 commercial seroconversion and performance panels. Results from 5 serially diluted positive patient samples were compared to results from the ORTHO® T. cruzi ELISA Test System on the ORTHO VERSEI® Integrated Processor. An endogenous interferent panel was also evaluated. Total within lab precision was evaluated over 20 days in accordance with CLSI EP05-A2 using 1 VITROS 3600 and 1 VITROS ECIQ Immunodiagnostic System.

Results: The assay specificity was 99.93% (1360/1361, 95%CI: 99.5% - 99.99%) for donor patient samples. Of the patient samples with unrelated medical conditions, 119 of the 122 non-Leishmania samples (97%) were nonreactive and 3 (2%) were repeatedly reactive. 14 of the 20 Leishmania infectious samples (70%) were found to be repeatedly reactive. The assay sensitivity was 100% (105/105; 95% CI: 96.55% - 100.0%) with the presumably positive patient panel. The seroconversion and performance panels were found to be reactive on both VITROS Systems at the same bleeds as all of the other commercially available assays. The VITROS T. cruzi Assay* generated similar dilutional performance as the Ortho T. cruzi ELISA Test System on both VITROS Systems. The assay showed no interference to hemolysis (<500 mg/dL), conjugated and unconjugated bilirubin (<20 mg/dL), total protein (<100 g/L), and triglycerides (<500 mg/dL). Within-lab precision of the assay ranged from 7.74 to 10.61 %CV above the cutoff (1.0-10 SC) and 10.06 to 12.7 %CV below the cutoff on both VITROS Systems.

Conclusion: The VITROS T. cruzi Assay* has been demonstrated to be sensitive and specific and has acceptable precision. Results are comparable to the ORTHO T. cruzi ELISA Test System, which received FDA approval in 2006.

Under Development
Can Medicaid and Medicare Claims Data be used for Influenza Activity Surveillance?

I. Fan1, r. astles2, h. burkom1. 1CDC, ATLANTA, GA, 2Johns Hopkins University, Baltimore, MD

Objective: To investigate the utility of claims data from the Centers for Medicare and Medicaid Services (CMS) as early indicators of increased influenza incidence. We evaluated diagnosis-based counts of selected claims data as influenza activity indicators.

Methods: We compared CMS claims data, including Medicare outpatient (OTP), physician office visits (Carrier) and Medicaid (MAX) data, with the National Respiratory and Enteric Virus Surveillance System (NREVSS) data from CDC as the “gold standard” for flu activity. Our statistical comparison used 2007-2012 claims data from 10 states, representing each HHS region. We calculated correlations of weekly time series of counts of CMS test orders and diagnosis counts with gold standard time series. Regarding the uncertainty of diagnosing influenza and seasonal expectations, we tried three separate case definitions to classify influenza-related CMS diagnoses: CD1=Specific code for influenza, CD2=CD1 or code for any influenza-like illness, CD3=CD2 or unspecified viral infection.

Results: Table 1 shows significant (p<0.01 throughout) correlation coefficients (CC) between CMS and gold standard time series, with bold font for values ≥ 0.7. All states’ OTP flu test count CCs exceeded 0.75. Diagnosis count CCs for CMS were less consistent, with OTP tracking the gold standard better than Carrier or MAX data. The OTP CCs exceeded 0.7 in nine of ten states for CD1 and in seven states for less specific CD2.

Conclusions: Correlations with gold standard influenza data support the surveillance utility of CMS flu test data when physicians are ordering tests. Diagnosis code count correlations based on influenza-like illness and unspecified infection codes indicate utility even when physicians do not expect or test for influenza. Understanding of differences in insurance coverage and reimbursement practices is needed to clarify state-specific utility. The approach for creating/validating case definitions is applicable to investigations of electronic health record data for surveillance of multiple disease outcomes.

<table>
<thead>
<tr>
<th>Data</th>
<th>CMS/gold standard correlation coefficients on weekly test count time series (NREVSS)</th>
<th>CMS/gold standard correlation coefficients based on diagnosis codes for (three CMS data types, three case definitions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carrier</td>
<td>OTP</td>
<td>MAX</td>
</tr>
<tr>
<td>CA</td>
<td>0.84</td>
<td>0.8</td>
</tr>
<tr>
<td>CO</td>
<td>0.82</td>
<td>0.8</td>
</tr>
<tr>
<td>FL</td>
<td>0.84</td>
<td>0.8</td>
</tr>
<tr>
<td>GA</td>
<td>0.85</td>
<td>0.8</td>
</tr>
<tr>
<td>MA</td>
<td>0.86</td>
<td>0.8</td>
</tr>
<tr>
<td>MI</td>
<td>0.86</td>
<td>0.8</td>
</tr>
<tr>
<td>OH</td>
<td>0.85</td>
<td>0.8</td>
</tr>
<tr>
<td>WA</td>
<td>0.85</td>
<td>0.8</td>
</tr>
<tr>
<td>WA</td>
<td>0.86</td>
<td>0.8</td>
</tr>
<tr>
<td>Total</td>
<td>0.84</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Prevalence and Genotype Distribution of Cervical Human Papillomavirus in Korean Women

Nah, S. Cho, H. Cho. Korea association of Heath Promotion, Seoul, Korea, Republic of

Background: Persistent infection of human papillomavirus (HPV) can cause the cervical intraepithelial lesions and carcinoma. The prevalence and genotype distribution of HPV infection have been known to be different among the geographical regions. For the development of strategies for prevention of cervical cancer, it is important to clarify the prevalence of HPV infection and genotype distribution in their own population. This study was performed to evaluate the prevalence and genotype distribution of HPV infection according to the age and cervical cytological findings in Korean women.

Methods: A total of 18,815 health examinees who were selected for this study, who took both tests of cervical cytology and HPV genotyping using multiplex PCR for screening of cervical cancer in 16 health promotion centers of 13 cities in Korea. The twenty-eight HPV genotypes were divided into two categories; high-risk HPV (carcinogen such as HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 58, 59, 69, and probable carcinogen such as HPV-68, 26, 53, 66, 70, 73, and 82), and low-risk HPV (HPV-6, 11, 40, 42, 43, 44, 51, and 61).

Results: The overall HPV prevalence was 27.8%, with 22.2% of high-risk HPV (HR-HPV), and 11.4% of low-risk HPV (LR-HPV). Among HR-HPV genotypes, HPV-53 was the most frequent (38%), followed by HPV-52(32%), HPV-70(32%), HPV-68(30%), HPV-58(27%), and HPV-16(29%). The prevalence of overall HPV, and HR-HPV infection increased along with the severity of cervical cytological findings (P for trend <0.001). The proportion of HR-HPV in relation with the age and cervical cytological findings, showed U-shape curve, with being the highest at age below 30 year-old, declining gradually down to the bottom at the age between 50-59, and then increasing afterwards in ASCUS/LSIL (P=0.001).

Prevalence and distribution of HR-HPV were different according to the age and cervical cytological findings, which provides a genotypic support for more effective screening of cervical cancer and appropriate vaccination.
HIV-1 and HCV sequence-based genotyping methods validated against TRUGENE commercial kits.


Background: The HCV and HIV-1 genotype determination have clinical importance because the response to antiviral treatment varies with the genotype. Furthermore, the classification of HIV-1 subtype and HCV genotypes provides a scientific opportunity to study the worldwide spread of these viruses. Sequence-based genotyping is the method that is most widely used to genotype. Some laboratories use in-house sequencing, but a number of commercial assays are also extensively used. Methods: Two genotyping assays were validated: one for HIV-1 and another for HCV genotyping in human plasma samples. For both assays, the viral RNA was extracted using the Abbott molecular m2000 sample preparation instrument. The HIV-1 genotyping assay included two regions of the HIV-1 virus RNA: env gp41 immunodominant and pol IN. The sequences were amplified by RT-PCR using specific primers for each one. The env gp41 immunodominant region was amplified using the forward primer, JH35F, and the reverse primer, JH38R. The sequences from the pol IN region were obtained using primers poli5_OF and poli8_OR, followed by a nested PCR using primers poli7_IF and poli6_IR. Samples with low viral loads (< 500 copies/ml) were analyzed using an alternative protocol designed to amplify the env gp41 immunodominant region. Reverse transcription and primary PCR were performed using primers GP40F1 and GP41R1. Primers GP46F2 and GP47R2 were then used for the nested PCR. The HCV genotyping assay was carried out using sequences obtained from the NS5b region. The sequences were amplified by RT-PCR using the specific primers Pr1 and Pr2. Alternatively a hemi-nested PCR was carried out using primers Pr3, Pr4 and Pr5. The obtained sequences were blasted against available HIV and HCV sequence databases. Results: Results were validated against previously obtained results from the TRUGENE HIV-1 genotyping kit and TRUGENE HCV 5'NC genotyping kit. All 20 samples (100%) for the HIV-1 subtypes showed concordant results in both methods. However, at sub-subtype level, one sample was not coincident showing an F sub-subtype with TRUGENE method and an F2 sub-subtype in this study. The HCV genotypes determined by the two methods were concordant in 100% (21/21) of the samples but the results differed at subtype level (15/21). On the other hand, the NS5b method was able to classify all samples at subtype level whereas the TRUGENE HCV 5’NC genotyping kit did not in four cases (20%). Conclusion: It is concluded that these methods are reliable and convenient for HIV-1 and HCV genotyping. Moreover, the HIV-1 genotyping assay permitted subtype identification in samples with a low viral load and the HCV genotyping assay showed more resolution at the subtype level in comparison with TRUGENE methods.
Infectious Disease

the control group (P<0.05) while the CRP in these patients showed no significant difference (P>0.05).

In viral infections, using CRP in conjunction with SAA improves diagnostic efficiency slightly but is significantly better than CRP alone. (AUC: SAA alone 0.83, SAA plus CRP 0.85, CRP alone 0.54).

Conclusion:

In most circumstances the serum concentration of SAA correlates well with that of CRP, but SAA reaches higher concentrations and may respond more rapidly. SAA results obtained by S-SPOT showed excellent discrimination between healthy adults and patients with viral and bacterial infections. Whole blood can be used on S-SPOT, saving the centrifugation step. S-SPOT is a reliable, rapid quantitative test which takes less than 3 minutes and is especially useful in pediatric care and emergency situations.

B-058

Circulating plasmablasts of chronic HIV-infected individuals produce polyreactive antibodies crossreactive with NMDAR and contribute to HAND and HAD

H. Liao, H. Jiang, J. Su. West China Hospital, Sichuan University, Chengdu, China

Background: In post-HAART era, the survival rate of individuals with HIV infection increased dramatically. Unfortunately, the prevalence of HIV-Associated Neurocognitive Disorders (HAND) and HIV-Associated Dementia (HAD) increased. Understanding the responsible mechanisms will help us find early diagnostic biomarkers and develop new therapies. Recent analyses of anti-gp120 antibodies showed that many of them display polyreactivities against different types of antigens. We hypothesize that the accumulated polyreactive antibodies in HIV patients may cross-react with neuronal antigens and contribute to HAND or HAD.

Methods: Single cell RT-PCR was performed to clone paired IgH and IgL genes from the plasmablasts of HIV-infected individuals and 80 recombinant monoclonal antibodies were cloned. Human neuroblastoma SH-SYSY cells were used as a model system to evaluate the antibodies effect on neuronal cells. ELISA analyses and immunofluorescence staining techniques were applied. 80 serum samples from chronic HIV-infected individuals were collected and tested for anti-NMDAR antibody titer and compared with controls.

Results: Over 60% of the HIV patient-derived antibodies cross-reacted with the DWDDS peptide, a dsDNA mimetope in NMDAR. 8 antibodies were able to bind to SH-SYSY cells. One such antibody, HIV201PBB2, can recognize the NMDARs on SH-SYSY cells. Co-culture of SH-SYSY cells with HIV201PBB2 resulted in clustering and internalization of NMDARs, decreased calcium influx and increased cell apoptosis, suggesting that HIV patient derived anti-NMDAR antibody can cause neuronal damage. Furthermore, HIV-infected individuals with manifestations of HAND/HAD showed higher titer of anti-NMDAR antibodies in serum.

Conclusions: That polyreactive antibodies generated by plasmablasts of HIV patients crossreact with NMDAR and affect neuronal cell function and survival may be a previously unrecognized mechanism of the pathogenesis of HAND and HAD. More clinical samples from AIDS patients with HAND/HAD will be involved to evaluate on whether such antibodies could serve as a new type of diagnostic biomarker for neuropathy, and therefore provide insight into new treatment target selections during chronic HIV infection.

B-057

Comparison of serologic Lyme testing with the Immunetics C6 ELISA, EUROMMUN EUROLINE-WB and Viramed Biotech ViraStripe assays


Objective:

The objective of this study was to compare the performance of the EUROMMUN Westenblot Anti-Borrelia Burgdorferi US EUROLINE-WB Ig assay with an integrated recombinant VlsE (variable major protein-like sequence, expressed) chip to that of the Immunetics® C6-B. burgdorferi ELISA™ Kit. A secondary goal was to determine if utilization of the VlsE antigen chip could replace the use of the current Immunetics C6 ELISA. Evaluation of the EUROMMUN Westenblot Anti-Borrelia Burgdorferi US EUROLINE-WB IgG and IgM assays compared with the Viramed® Biotech AG - Borrelia B31 IgG and IgM ViraStripe® assays was also performed.

Methodology:

The Immunetics C6 ELISA (Immunetics, Inc., Boston, MA) utilizes a synthetic C6 peptide, derived from the VlsE protein of B. burgdorferi, bound to the wells of a microwell plate. Antibodies in the patient serum are captured by the immobilized antigen and then detected by the addition of a horseradish peroxidase-conjugated goat anti-human IgG/IgM conjugate. Incubation with a chromogenic peroxidase substrate results in color development, which is then read via a spectrophotometer.

The EUROMMUN EUROLINE-WB IgG and IgM assays (EUROMMUN AG, Luebeck, Germany) contain test strips with antigen extracts of B. burgdorferi. Test strips also contain a membrane chip coated with B31 antigens and recombinant VlsE antigen (IgG). Antibodies present in the patient serum bind to the antigens on the strip and are detected using an enzyme-labeled anti-human IgG/IgM which in the presence of substrate results in color development. The Viramed Biotech IgG and IgM ViraStripe assays (Viramed Biotech AG, Planegg/Steinkirchen, Germany) contain specific B31 antigens that are bound to a solid phase nitrocellulose support membrane. Antibodies present in the patient serum bind to the antigens on the strip. Enzyme labeled anti-human IgG/IgM is added to detect bound antibody. Substrate is added which undergoes a color change.

Results:

Two-hundred and forty-nine serum specimens were available for testing by all methods. Comparison between the Immunetics C6 ELISA and the EUROMMUN EUROLINE-WB IgG integrated VlsE chip assay demonstrated an overall agreement of 91.7% with 96.8% of the positive specimens and 90.0% of the negative specimens demonstrating agreement between the methods. The Immunetics ELISA called more specimens positive while the EUROMMUN assay generated a greater number of borderline results. These same specimens were also analyzed by the Viramed Biotech IgG and IgM ViraStripe assays and the EUROMMUN EUROLINE-WB IgG and IgM assays. An overall agreement of 85.1% was observed between the IgG WB assays while the IgM WB assays gave an overall agreement of 76.3%. Both WB assays demonstrated good negative agreement at 95.2% and 98.8% for IgG and IgM, respectively with lower rates of positive agreement at 54.8% and 36.0%, for IgG and IgM, respectively.

Conclusion:

Findings from this study demonstrate that testing performed utilizing a recombinant VlsE chip is generally comparable to that provided by current screening assays. The assays employed for WB analysis demonstrate good agreement when ruling out Lyme disease, however confirmation of diagnosis via positive results was less definitive.

B-059

Incidence study of sexually transmitted infections in asymptomatic patients and association with cervical intraepithelial neoplasia.

L. M. Lima1, C. Hoelzle2, J. R. B. Fradico2, R. T. Simões1, L. M. Lima1, E. Mateo1, D. A. G. Zaulli1, V. H. Melo2. 1Instituto de Ensino e Pesquisa da Santa Casa, Belo Horizonte-MG, Brazil, 2Fundação Oswaldo Cruz, Belo Horizonte-MG, Brazil, 3Herpes Pardini Institute (Research and Development Sector), Vespasiano, Brazil, 4Faculty of Medicine of UFMG, Vespasiano, Brazil

Cervical carcinoma is the third most common cancer among women worldwide and most cases in developing countries. The carcinogenic human papillomaviruses (HPV), a highly prevalent sexually transmitted infection (STI), is a necessary cause for the development of cervical intraepithelial neoplasia (CIN) and cervical carcinoma. However, among women infected with high-risk HPV, only some will develop CIN/ cervical carcinoma, suggesting that carcinogenic process following HPV infection and CIN is likely influenced by biological cofactors, such as Chlamydia trachomatis, Neisseria gonorrhoeae, Ureaplasma urealyticum, Mycoplasma hominis, and Mycoplasma genitalium infection that are often asymptomatic infections. Epidemiological studies about the impact of these infections on HPV acquisition and development to CIN or cervical carcinoma have yielded equivocal results due to the difficulties in separating biological from behavioral effects. The aim of this study was to investigate the incidence of HPV, C. trachomatis, N. gonorrhoeae, U. urealyticum, M. hominis, and M. genitalium in sexually active women and to evaluate the association these infections with CIN. A total of 126 patients was subjected to collection of cervical cytology. The samples (vaginal swabs) were examined by Real Time PCR and TaqMan detection system with specific primers and probes for each infectious agent. For the statistical analysis, the chi-square test was used considering the 0.05 significance level, and a multivariate analysis was performed by logistic regression. The results obtained showed that incidence of STIs was HPV 46.8%, C. trachomatis 27.8%, M. genitalium 28.6%, M. hominis 0.8%, U. urealyticum 4.8%, N. gonorrhoeae
Wednesday, August 3, 9:30 am – 5:00 pm

4.8%. HPV (p = 0.024), C. trachomatis (p = 0.009), M. genitalium (p = 0.040) infection. Coinfection with HPV and C. trachomatis (p = 0.023), and HPV infection associated with the presence of at least one STI (p = 0.011) were associated with CIN. After multivariate analysis, a positive association was found between HPV and CIN (p = 0.040, OR = 2.48, 95% CI: 1.04-5.92) and between C. trachomatis and CIN (p = 0.028, OR = 2.69, 95% CI: 1.17-6.53). Therefore, in our study, HPV, Chlamydia, M. genitalium infection and coinfection with HPV and Chlamydia and HPV infection associated with the presence of at least one STI constitute significant risk factors for the occurrence of CIN. Although these findings did not significantly differ by HPV genotype, the high frequency of these infections in asymptomatic women confirms that the inclusion of a diagnostic screening for detection of HPV in patients with STIs seems to be relevant in clinical and laboratory routine.

B-060
Comprehensive Clinical Evaluation of BacT Alert 3D Resin Bottles for Blood Cultures
S. Kim1, J. Byun2, W. Choi1. 1Changwon Gyeongsang National University Hospital, Changwon, Korea, Republic of; 2Gyeongsang National University Hospital, Jinju, Korea, Republic of; 3Kyungsung University, Busan, Korea, Republic of

Background: We prospectively evaluated the performance of blood culture resin media: FA Plus and FN Plus of BacT Alert 3D System (bioMerieux) in a tertiary university-affiliated hospital in 2014. Resin may enhance the growth of microorganisms by adsorbing antibiotics in the blood sample. Positive and time to detection (TTD) is the key indicator to assess the performance of blood culture system. TTD is defined by the time interval from the entry to the automatic blood culture machine to the positive signal of microorganism.

Methods: We obtained 2,994 blood culture sets. Ten ml of venous blood were collected for each puncture and distributed into each bottle evenly. Blood culture media were immediately entered in the BacT Alert 3D System. We categorized the bottles into two groups based on the prior antibiotic treatment. Terminal subculture was performed for the signal-negative bottles after 5 days of incubation to evaluate the false negative in the blood culture machine. Positive and TTD of microorganisms were analyzed for the clinically significant microorganisms.

Results: Of 2,994 sets received, 371 sets (12.4%) yielded 385 clinically significant pathogens. There was no statistically significant difference between prior antibiotic users (13.1%, 65/498) and antibiotics naïve patients (12.3%, 306/2,496) (P=0.05) in the positivity. Growth was observed in 0.35% (9/2,623 sets) in the terminal subculture. Staphylococcus aureus, gram-positive cocci, and all microorganisms were detected significantly faster in FA Plus (13.81 h, 14:09 h, and 11.12 h, respectively) than in FN Plus (14.82 h, 15:60 h, and 11.88 h, respectively). Otherwise, there was no significant difference in TTD for Gram-negative rod and yeasts. The distribution of the TTD was noteworthy. Over 88% of positive signals were encountered in the first 24 h. More than 97% were detected in both FA and FN bottles within 48 h. Within 72 h, 99.7% were detected in FA Plus and 99.0% in FN Plus.

Conclusions: Detection of microorganisms was equally complementary between FA Plus and FN Plus. Gram-positive cocci including S. aureus grew earlier in FA Plus. The positive rate was not affected by prior antibiotic therapy in BacT Alert 3D resin media. False-negative in the machine seems negligible. Since 99.0% of TTD were detected within 72 hours, laboratories with limited resources could apply 3 day incubation protocol instead of current 5 day incubation.

B-061
Evaluation of Performance of Enzyme Linked Fluorescent Assay, Multiplex PCR and Real time PCR for Detection of Clostridium difficile
S. Kim1, J. Byun2, W. Choi1. 1Changwon Gyeongsang National University Hospital, Changwon, Korea, Republic of; 2Gyeongsang National University Hospital, Jinju, Korea, Republic of; 3Kyungsung University, Busan, Korea, Republic of

Background: Clostridium difficile causes antibiotic-associated diarrhea and pseudo-membranous colitis. C. difficile generates toxin A (enterotoxin) and toxin B (cytotoxin) which are the major factors of C. difficile associated diarrhea (CDAD). These toxins or toxin genes are the targets for the laboratory diagnosis of CDAD. We evaluated the performance of variable detection methods of C. difficile.

Methods: We obtained 354 clinical fecal specimens which were requested for C. difficile toxin tests in 2014. The enzyme linked fluorescent assay (ELFA), VIDAS C. difficile Toxin A & B (VIDAS CDAB, bioMerieux, France) was used for the toxin tests. Toxin B gene was detected using multiplex PCR, Seeplex Diarrhea ACE Detection panel B1 (Seegene, Korea) and both toxin A and toxin B genes were detected by Advansure CD real time PCR (LG Life science, Korea). Positive rate and concordance rate of each tests were compared.

Results: Among 354 stool specimens, 38 (11.4%) were positive by VIDAS assay. 60 (16.9%) by PCR, and 82 (23.2%) by real time PCR. In comparison of real time PCR with VIDAS, positive concordance rate was 100%, negative concordance rate was 89.1% (P<0.001), and discordance rate was 9.7%. Between PCR and VIDAS results, positive concordance rate was 84.2%, negative concordance rate was 93.4% (P<0.001), and discordance rate was 7.6%. When real time PCR was compared with PCR, positive concordance rate was 93.3%, negative concordance rate was 91.2% (P<0.001), and discordance rate was 8.5%.

Conclusion: Comparative evaluation of three methods showed that real time PCR was a relatively appropriate test to detect C. difficile since it is fast and convenient with the highest detection rate. Real time PCR and VIDAS showed the highest concordance rate.

B-062
Quantification of CSF chemokines and cytokines allows for rapid laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens
D. Fortuna1, A. M. Cardenas2, E. H. Graf2, A. L. Roberts1, L. Harshyne1, D. C. Hooper1, M. T. Curtis1. 1Thomas Jefferson University Hospital, Philadelphia, PA; 2Children’s Hospital of Philadelphia, Philadelphia, PA

Background: Prompt diagnosis of central nervous system (CNS) disease is critical to guide intervention and appropriate therapy. Development of novel laboratory approaches to rapidly classify acute-onset CNS disease is in great demand. Serious microbial pathogens, especially viruses, are quickly expanding beyond their historic geographic range and may not even be considered in the clinician’s differential diagnosis. Unlike bacterial cultures, current viral testing targets a limited number of viruses. Additionally, despite diversity in etiology, signs and symptoms of both infectious and non-infectious CNS disorders can be remarkably similar, which can confuse the clinical picture and delay treatment. Bacterial, viral, fungal and parasitic CNS pathogens are sensed by pattern recognition receptors of the immune system, stimulating immediate release of measurable levels of chemokines and cytokines into the CSF. Our objective is to use pathogen-specific chemokine/cytokine profiles to classify CNS disease as infectious versus non-infectious and further discriminate between viral and non-viral infections.

Methods: Levels (pg/ml) of chemokines and cytokines were determined in the CSF of 45 patients with documented infectious meningitis or meningoencephalitis (mean age 19.2 years) and in the CSF of 25 patients who were negative for CNS infection (mean age 27.4 years). MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panels (Millipore) were used to measure CSF chemokines and cytokines levels (pg/ml). Immune analyte quantification included IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L. Samples were analyzed in duplicate by a FlexMAP 3D (Luminex). Standard curves were generated for each cytokine and median fluorescent intensities were transformed into concentrations by 5-point, non-linear regression. For univariate analysis, comparisons between groups were made using the Mann-Whitney test. We utilized receiver operating characteristic (ROC) curve analysis to calculate areas under the ROC curve (AUC) for each analyte to access the utility of chemokine/cytokine levels as discriminating tests. The ROC generated sensitivity and specificity values were then used to determine clinically optimal cutoff values for the informative analytes.

Results: Univariate analysis utilizing Mann-Whitney tests demonstrated that median values (pg/ml) of IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L were all significantly higher in CSF from patients with infectious brain disorders than in CSF from patients with non-infectious disorders (p-value < 0.05). MDC (CCL22) demonstrated statistical significance, when comparing viral infections versus non-viral infections (with the non-viral infection group having higher analyte levels). IP10 (CXCL10) can reliably distinguish between an infectious versus non-infectious CNS process (AUC 0.9778) with an optimal cut-off value of 2023 pg/ml (sensitivity, specificity; 91.67%, 87.88%). In the infectious group, MDC (CCL2) can reliably differentiate between viral and non-viral infections.

Conclusion: Recent advances in the laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens offer promise in the rapid diagnosis of CNS infections. Further work is needed to validate these platforms in clinical practice.

S160 68th AACC Annual Scientific Meeting Abstracts, 2016
Infectious Disease

and non-viral CNS infections. These results suggest that CSF chemokine/cytokine quantification can serve as a useful laboratory tool for the rapid triage of CNS diseases to help guide prompt therapy and further testing.

**B-063**

Next Generation Sequencing-based HIV-1 Drug Resistance Monitoring System

E. Rakhamianlev,1 P. Ariyaratne,1 P. Nisa,1 Suniwong2, C. Wathiphan2, Z. Rui1, E. Passomub2, W. Chantratit1, G. Michel1. Vela Diagnostics, Singapore, Singapore, 1Department of Pathology, Faculty of Medicine, Ramathibodi Hospital Mahidol University, Bangkok, Thailand

**Background:** The most common cause for therapeutic failure in people infected with Human Immunodeficiency Virus (HIV) is the resistance of HIV to antiretroviral drugs. Objective of this study was to compare two sequencing-based HIV-1 drug resistance monitoring systems: an CLIP-based system (TruGene HIV-1 Genotyping Kit) and a novel Next Generation Sequencing (NGS)-based test (Sentosa SQ HIV-1 Genotyping Assay).

**Methods:** We used a newly developed automated NGS-based integrated workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for nucleic acid extraction and NGS library preparation (Sentosa SX101); 2) Ion Torrent instruments for template preparation and deep sequencing; 3) kits for RNA extraction, HIV NGS library preparation, template preparation and deep sequencing, and 4) data analysis and reporting software. Reporting includes 86 Drug Resistance Mutations (DRMs) across the Reverse Transcriptase (RT), Protease (PR) and Integrase genes. 111 prospective EDTA plasma clinical samples from patients infected with HIV-1 were tested for this study.

**Results:** All 111 HIV-1 positive samples were tested on both systems. 97.3% (108/111) samples were subtyped as CRF01_AE. In total, 647 DRMs were detected (435 in the RT gene, 199 in the PR gene and 13 in the Integrase gene). The Sentosa SQ HIV Genotyping Assay detected 100% (199/199) of all DRMs in the PR gene and more than 98% DRMs (427/435) in the RT gene. The Integrase gene was not included into the comparison study because it is not covered by the TruGene test. In total, 130 DRMs were detected by the Sentosa SQ HIV Genotyping Assay, that were not found by TruGene and 8 DRMs were missed by the Sentosa HIV Genotyping Assay (but detected by TruGene). Mutation detection rate for the HIV PR gene was 100% (95%CI: 98.11-100%) for the Sentosa SQ HIV Genotyping Assay and 90.45% (95%CI: 85.57-93.80%) for the TruGene system. In the RT gene the 98.16% (95%CI: 96.41-99.07%) of DRMs were recorded by the Sentosa SQ HIV Genotyping Assay and 74.48% (95%CI: 70.18-78.75%) by TruGene. Overall DRM detection rates aggregated were 98.74% (95%CI: 97.53-99.36%) for the Sentosa SQ HIV Genotyping Assay and 79.5% (95%CI: 79.02-79.62%) for the TruGene HIV-1 Genotyping Kit. All HIV strains were carrying 1 or multiple DRMs in RT, 16, 19 and 9 AA positions of the RT, PR and Integrase genes respectively. The most prevalent DRMs in the RT gene were: M184V was present in 48.7% (54/111) of the samples, K103N in 29.7% (33/111), Y181C in 27.9% (31/111), G190A and D67N (both 18.9% (21/111)). In the PR gene: M36I 91.9% (102/111), K20R 61.6% (24/111) and L10I 20.7% (23/111).

**Conclusion:** Timely detection and reporting of DRMs is critical for drug regiment and can minimize the development of resistance to antiviral drugs. In this perspective the NGS-based workflow appears as a promising new tool for detecting clinically relevant variants in HIV-1. Given its high sensitivity (up to 5% mutation frequency) compared to Sanger sequencing-based systems and the comparatively short turnaround time of 2.5 days the workflow provides comprehensive, clinically relevant information for optimal selection of HIV-1 treatment regimens.

**B-065**

A qPCR assay that simultaneously detects Mycoplasma genitalium and mutations associated with macrolide resistance has the potential to improve patient management

A. V. Todd1, L. Tan2, S. M. Walker1, E. Mokany2, J. Su3, C. S. Bradshaw4, C. K. Fairley1, S. M. Garland1, S. N. Tabrizi5, 1SpeexDX PTY LTD, Sydney, Australia, 2The Royal Children’s and The Royal Women’s Hospitals, Melbourne, Australia, 3Melbourne Sexual Health Centre, Melbourne, Australia

**Background:** In the 2015 CDC STD treatment guidelines, Mycoplasma genitalium (Mg) was highlighted as an emerging issue based on increasing evidence linking it with nongonococcal urethritis (NGU) in men and cervicitis in women. However, to-date diagnosis has been difficult as it is a slow growing organism and can take up to 6 months to culture. It has only been with the advent of NAAT testing that routine detection has been made possible. Further complications have arisen by emerging resistance to the standard treatment for Mg which is a single dose of 1g azithromycin, a macrolide antibiotic. We report the use of a multiplexed qPCR assay for the detection of macrolide resistance and ineffective cure rates. A new assay, PlexPCR™ M. genitalium ResistancePlus kit, has been developed to simultaneously identify Mg and 5 mutations in the 23S RNA gene (positions 2058 and 2059 (E. coli numbering)) associated with macrolide resistance. The assay has previously been demonstrated to have good clinical performance on 400 retrospective samples (sensitivity and specificity for Mg and 23S rRNA mutation detection was 99.1% and 98.5%, and 97.4% and 100%, respectively). This study evaluates incorporating the assay into a diagnostic algorithm to direct faster and more appropriate clinical management and reduce the spread of antibiotic resistant Mg by testing for resistance upfront.

**Methods:** In this prospective study, 1087 consecutive urogenital samples from symptomatic and asymptomatic patients were evaluated with the PlexPCR M. genitalium ResistancePlus kit. This is a real-time PCR kit that employs novel PlexPrimer and PlexZyme technology, PlexPrimers selectively amplify mutants over wild-type and PlexZymes allow for efficient multiplexed detection and signalling. This
unique combination allows “stacking” of the 5 mutation assays for a single readout. This kit was run in parallel to an in-house test for Mg detection and sequencing of Mg positives to determine 235 rRNA mutation status.

**Results:** The prevalence of Mg was 6.0% (65/1087) and in the Mg positive samples 235 rRNA mutation prevalence was 63.1% (41/65). The PlexPCR M. genitalium ResistancePlus assay showed very high clinical performance compared to the reference methods with sensitivity and specificity for Mg detection of 98.5% (95%CI: 91.7-100%) and 100.0% (95%CI: 99.6-100%), and 235 rRNA mutation detection of 92.7% (95%CI: 80.1-98.5%) and 95.7% (95%CI: 78.1-99.9%) respectively. The PlexPCR M. genitalium ResistancePlus assay showed a limit of detection (LOD) of 10 - 15 copies for each mutant when LOD study was performed on synthetic template and no cross-reactivity of related organisms was seen.

**Conclusion:** The PlexPCR M. genitalium ResistancePlus kit demonstrated excellent clinical performance for the simultaneous detection of Mg and assessment of macrolide resistance. Performance of this assay as a rapid screening assay can offer better clinical management of macrolide resistant Mg infection.

**B-066**

Performance Evaluation of a Prototype CMVIgG Assay on the ADVIA Centaur® System

R. Martin, R. Stadnick II, W. Zazulak, K. Freeman, M. Dobbins, M. Mansini, S. Patibandla. Siemens Healthcare, Tarrytown, NY

**Objective:** Siemens Healthcare (Tarrytown, NY) is currently developing a cytomegalovirus IgG (CMVIgG) assay to detect the presence of IgG antibodies to cytomegalovirus (CMV). Anti-CMV IgG antibodies act as a specific marker to aid in the diagnosis of CMV infection. Changes in the seroconversion status of CMV IgG are an indicator of either a reactivation or reactivation of CMV. The Siemens ADVIA Centaur® CMVIgG assay* is a chemiluminescent monoclonal microparticle-based immunoassay that utilizes the NSP-DMAE molecule and runs on the ADVIA Centaur Immunoassay System. The objective of this study was to evaluate the positive and negative agreement, precision, and cross-reactivity of a prototype, automated ADVIA Centaur CMVIgG assay.

**Methods:** The fully automated ADVIA Centaur CMVIgG assay is being developed as an indirect sandwich assay for the detection of CMV IgG antibodies in human serum and plasma. The assay was evaluated for positive and negative agreement (via method comparison), cross-reactivity, repeatability, and within-lab precision. The positive and negative agreements of the assay were evaluated using a total of 2315 patient samples across two reagents lots. Cross-reactivity was evaluated using samples negative for CMV IgG but known to have at least one of the following disease states: Epstein-Barr virus (EBV), herpes simplex virus (HSV), toxoplasma, Rubella, Chlamydia, measles, Varicella Zoster Virus (VZV), Rheumatoid Factor (RF), Hepatitis C virus (HCV), and Hepatitis A virus (HAV). The results were assessed based on index values as reactive (≥1.00) or nonreactive (<1.00). The serological status of all samples was initially determined by the bioMerieux Vidas® CMV IgG assay. Discordant samples were tested on the Siemens IMMULITE® 2000 and Roche cobas e 411 CMV IgG assays, when available. Per CLSI EP5-A3, precision was evaluated by testing four samples with index values concentrations spanning the assay range in two runs per day for 20 days on the ADVIA Centaur system for a total of 80 replicates.

**Results:** Evaluation of the patient samples using the ADVIA Centaur CMVIgG assay indicated that the positive agreement ranged from 98.7% to 99.4% and the negative agreement ranged from 99.5% to 99.9%, when compared to the VIDAS CMV IgG assay. Additionally, the ADVIA Centaur CMVIgG assay displayed a total agreement of 99.3% (133/134) to the resolved clinical status of all CMV IgG-negative cross-reactive samples evaluated. The assay demonstrated good precision, with an average agreement ranging from 98.5% to 99.5%, when compared to the VIDAS CMV IgG-negative agreements of the assay were evaluated using a total of 2315 patient samples

**Conclusion:** The results of this study demonstrate good performance of the prototype ADVIA Centaur CMVIgG assay. *For investigational use only. Not available for sale. The performance characteristics of this product have not been established.

**B-067**

Heparin Binding Protein: a potential new biomarker for diagnosis of Acute Bacterial Meningitis

M. H. Kandil1, G. I. Khalili1, E. A. El-Attar2, S. Hassan3. 1Medical Research Institute, Alexandria University, Alexandria, Egypt, 2Fever Hospital, Alexandria, Egypt

**Background:** Acute bacterial meningitis is a life-threatening neurological emergency. It is one of top 10 causes of infection-related death worldwide. Untreated, its mortality approaches 100%. Even with current antibiotics and advanced intensive care, the mortality rate of the disease is approx. 10% & 30-50% of its survivors have permanent neurological sequelae. The clinical distinction between viral & acute bacterial meningitis is difficult in the acute phase of illness because the symptoms often are similar. Cerebrospinal fluid (CSF) examination is essential to establish the diagnosis & to identify the etiological agent. Bacterial culture is the gold-standard technique for confirmation. However, approx 50% of suspected cases are not culture-confirmed. It is also not uncommon that antibiotics are instituted before lumbar puncture, reducing the chance of a microbiological diagnosis. A rapid noninvasive biomarker for diagnosis & differentiation of ABM from is much needed. Heparin-binding protein (HBP) forms a part of the innate defenses of human neutrophils. HBP is rapidly mobilized from migrating neutrophils. **Methods:** 90 Egyptian individuals divided into three groups; 30 patients diagnosed with ABM, 30 diagnosed with patients with aseptic meningitis & 30 subjects with normal CSF examination findings (controls). Diagnosis was based on history, clinical criteria & CSF examination; macroscopic examination, microscopy (CSF cells and Gram stain), chemical examination (CSF Protein, glucose and lactate), latex agglutination & culture and sensitivities. HBP was measured using ELISA technique in both serum & CSF. **Results:** CSF HBP levels in the controls averaged 0.82 ± 0.3 ng/mL. In viral meningitis, mean CSF HBP levels 3.3 ± 1.7 ng/mL. In bacterial meningitis, mean CSF HBP levels was 174.8 ± 46.7 ng/mL. The mean serum HBP levels in the controls was 0.84 ± 0.3 ng/mL. In viral meningitis, mean serum HBP levels was 3.7 ± 1.9 ng/mL. In bacterial meningitis, mean serum HBP levels was 192.2 ± 56.6 ng/mL. CSF and serum HBP levels were significantly higher in patients with ABM than in patients with viral meningitis and controls. A cut-off of CSF HBP level of 56.7 ng/mL and serum HBP level of 45.3 ng/mL showed 100% sensitivity, specificity & positive & negative predictive values of 100% and overall accuracy of 100%. Area under the ROC curve for HBP was 1.0. Even in ABM patients who received antibiotics 48 - 72 hours prior to lumbar puncture, HBP levels in CSF & serum remained elevated. A significant positive correlation between CSF and Serum HBP levels and other CSF findings in all three groups. **Conclusion:** HBP levels in CSF & serum serve equally as strong potential diagnostic markers for bacterial meningitis and differentiation between bacterial and viral meningitis. HBP might be able to assist in early identification of bacterial meningitis even in empirically partially treated bacterial meningitis cases.

**B-068**

The Prevalence of Six Common Sexually Transmitted Pathogens by Multiplex Real-Time PCR in Cervico-Vaginal Specimens Collected from Sexually Active Women in Korea

A. Lee, J. Park, D. Kim, A. Cho, Y. Kim, K. Lee. Seoul Clinical Laboratories, Seoul Medical Science Institute, Yongin-si, Korea, Republic of

**Background:** Sexually transmitted infections (STIs) present a major public health concern worldwide. Reduction of complication rates and disease spread requires the use of rapid and reliable laboratory diagnostic tool that can identify causative pathogen. Multiplex real-time PCR assay is a highly sensitive and effective diagnostic tool for detection of urogenital pathogens, especially for culture-difficult organisms. The objective of this study was to assess the prevalence of six common sexually transmitted pathogens and the co-infection rate by multiple pathogens in female genital tract using a multiplex real-time PCR assay. **Methods:** The authors retrospectively analyzed the STIs multiplex real-time PCR results from 3,460 women (mean age: 36±7.10 years). STIs tests were requested to Seoul Medical Science Institute from 43 medical institutions nationwide in Korea between January 2015 and September 2015. Multiplex real-time PCR assays were performed with cervico-vaginal swabs by use of AccuPower® STIA-8plex Real-Time PCR Kit (Bioneer, Korea). Detection of the six types of STI pathogens including *Mycoplasma hominis* (MH), *Ureaplasma urealyticum* (UU), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Trichomonas vaginalis* (TV), and *Neisseria gonorrhoeae* (NG) was performed simultaneously in a single multiplex real-time-PCR reaction using a combination of primers for each pathogen. **Results:** Of the 3,460 subjects, 1,266 (36.6%) tested positive for at least 1 pathogen, with 836 (24.2%) positive for 1 pathogen, 337 (9.7%) positive for 2 pathogens, 173 (5.1%) positive for 3 pathogens, 94 (2.7%) positive for 4 pathogens, 43 (1.3%) positive for 5 pathogens, and 2 (0.1%) positive for 6 pathogens.
The utility of QuantiFERON-Gold in Tube assay to diagnosis of active tuberculosis: Using new equation

M. Kim, S. Kang, W. Lee, Y. Jeon. Kyung Hee University Hospital at Gangdong, Seoul, Korea, Republic of

Objective
Interferon-γ release assay (IGRA) measure a cellular immune response to Mycobacterium tuberculosis (Mtb) specific antigens. QuantiFERON-Gold in Tube assay (QFT-GIT) is a kind of interferon-γ release assay (IGRA) for the detection of Mycobacterium tuberculosis (Mtb). QFT-GIT is known to be highly specific and sensitive but cannot distinguish active from latent tuberculosis (TB) infections. The purpose of this study was to evaluate a new equation using interferon-γ (IFN-γ) levels for diagnosis active TB in positive QFT-GIT population.

Method
During the period from January 2013 to May 2015, 295 cases with positive QFT-GIT and having final diagnosis of active TB or non-TB by work-up including liquid cultures, TB polymerase chain reaction (TB-PCR), or acid fast staining were retrospectively studied. We compared individual IFN-γ levels and new equation between TB and non-TB. The IFN-γ levels were recalculated by standard curve and used without treatment in case with higher than 10 IU/mL. The characteristics of IFN-γ responses were analyzed by statistics including Mann Whitney U-test, Pearson’s Chi-square test and ROC curve analysis.

Results
The number of active TB and non-TB was 144 (age 51.5±19.0, M:F=86:58) and 151 (age 63.5±14.2, M:F=98:53), respectively. The IFN-γ levels of Mitogen and Nil were inversely correlated with age but not in TBAg tube. The cut-off of INF-γ in Nil tube were inversely correlated with age but not in TBAg tube. The active TB showed sensitivity 57% and specificity 80%.

Conclusion
The maintenance of HIV-1 genotyping programs is important in the diagnosis, monitoring, therapy and development vaccines. Subtyping is a powerful molecular tool for the choice of an effective therapy and monitoring the evolution of the HIV-1 epidemic. In Brazil, the majorities of published data about the prevalence of HIV-1 are from south and southeast remain scarce in other regions. Objective: To estimate the prevalence of HIV-1 subtypes and resistance profiles in order to contribute to the implementation of public health policies in Brazil. Method: Plasma samples from 184 diagnosed HIV-infected patients of different geographical regions of Brazil with viral loads above 1,000 copies/mL were used. Viral cDNA fragments corresponding to the reverse transcriptase and protease regions were amplified and sequenced. The subtype and mutations profile were assessed using Stanford HIV Drug Resistance Database.

Results: The mean age of the patients was 37.17 (range 4-74 years), and most samples were from men (80.43%). Among the 184 patients, 144 (78.26%) presented mutation in the analyzed region (21.74%), of which 96 (52.17%) to NRTIs, 104 (56.52%) to NNRTIs and 82 (44.57%) to protease inhibitors. According to analyses, a variety resistance mutations in Brazil, updated 2015
Distribution and Phenotypic Resistance Profile from Bloodstream Infections Isolates from Metropolitan Public Hospitals of Sao Paulo City, Brazil: Data from a Laboratory of Clinical Microbiology

J. Monteiro, F. M. Inoue, D. R. Ramadan, A. P. T. Lobo, M. C. Feres, S. Tuflik. Associação Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil

Background: Nosocomial bloodstream infections (BSI) are directly related with high morbidity and mortality rates, hospital lengths of stay in critical ill patients mostly and associated health care costs. Brazilian studies concerning bloodstream infections are important tools that can bring specific issues related to antimicrobial resistance. The aim of this study was evaluate the frequency of microorganism isolation and the main phenotypic resistance profiles from BSI in medical centers located throughout in the city of Sao Paulo, Brazil. Methods: Data on bacterial and fungal isolates from BSI patients admitted to the 10 public health system medical centers were selected from July 2014 to July 2015. Identification and susceptibility tests were performed using Vitek MS and Vitek 2 (bioMerieux) systems, respectively. Antimicrobial susceptibility profile were evaluated according CLSI, 2014/2015 criteria. Coagulase Negative Staphylococcal (CNS) BSI was only considered in the presence of at least two sets of positive blood cultures with the same organism. Results: A total of 8632 microorganisms were evaluated. The frequency of organism group type were Gram-positive (n=4841; 56.1%), Gram-negative (n=3539; 39.8%), yeasts (n=400; 4.6%), and fungi (n=172; 2%). Concerns persist about the variable predictive value of RIDTs, within the context of the prevalence of circulating influenza viruses in the population being tested. This is of particular concern in outpatient and ambulatory settings where RIDTs are often the only readily-available influenza tests. Methods: The SIRAS course was originally offered in Oct. 2012, and restructured in 2013 from a single to four 30- minute modules (http://www.jointcommission.org/siras.aspx). The new influenza pandemic preparedness course, “Influenza Preparedness and Response in Ambulatory Settings (SIRAS)”, with continuing education credits (CEs). The course focuses upon RIDT utilization. Further objectives were to gather feedback from course participants about SIRAS and other course e-resources and increased course enrollments. YouTube specimen collection videos, modules before the onset of influenza season specifically attracts high usage of the e-resources and increased course enrollments since its original launch in Apr. 2015 through Sept. 2015. Since updating to the SIRAS webpage and 1,615 enrollments in SIRAS between Oct. 2014 and Sept. 2015. Currently being translated into Spanish.

Table 1: SIRAS Course Usage & Enrollments

<table>
<thead>
<tr>
<th>Course Module</th>
<th>SIRAS Course Usage</th>
<th>SIRAS Course Enrollments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Collection</td>
<td>1,356,763 views</td>
<td>1,582 enrollments</td>
</tr>
<tr>
<td>RIDT Utilization</td>
<td>1,267,345 views</td>
<td>1,412 enrollments</td>
</tr>
<tr>
<td>Influenza Testing</td>
<td>1,154,987 views</td>
<td>1,301 enrollments</td>
</tr>
<tr>
<td>Preparedness</td>
<td>1,042,875 views</td>
<td>1,189 enrollments</td>
</tr>
<tr>
<td>Overall</td>
<td>5,864,880 views</td>
<td>7,603 enrollments</td>
</tr>
</tbody>
</table>

Methods:
The objective of this study was to assess the sample-to-sample contamination rate to carbapenem was one of the most higher when compared with others Gram-negative. Rates of antimicrobials resistance reported in CNS, Enterococcus spp. and K. pneumoniae are reinforce the high resistance rates to antimicrobials in Sao Paulo city that is considered one of the highest observed in Brazil. A continued study is necessary to monitor these national trends.
Performance Evaluation of Prototype Herpes-I IgG and Herpes-II IgG Assays* on the ADVIA Centaur XP Immunossay System

S. Patibandla, Siemens Healthcare, Tarrytown, NY

Background: Two members of the herpes virus family, herpes simplex virus 1 and 2 (HSV-1 and HSV-2), are pervasive, and both infect epithelial cells. While HSV-1 produces most cold sores, HSV-2 produces genital herpes. The ADVIA Centaur® Herpes-I IgG and Herpes-II IgG assays* from Siemens Healthcare are indirect sandwich chemiluminescent two-step immunoassays that employ the recombinant HSV-1 and HSV-2 glycoprotein G (gG1 and gG2) respectively. They are designed for the detection of HSV-1 and HSV-2 antibodies in order to allow a prompt recognition of herpes simplex infection and early initiation of therapy. The aim of this study was to evaluate the performance of the assays on the ADVIA Centaur XP Immunossay System.

Methods: Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the Siemens Herpes-I IgG and Herpes-II IgG assays were assessed by testing on 970 serum and 670 plasma samples against LIAISON (DiaSorin) and HerpeSelect 1 and 2 Immunoblot IgG (Focus) assays, respectively. Samples came from the Centers for Disease Control (272 sera), from different commercial sources (75 samples), from sexually active adults provided by ProMedDx (Norton, U.S.) and Cerba (Spain) (367 sera), and from pregnant women, also acquired from ProMedDx (Norton, U.S.) (228 sera). Other relevant performance characteristics such as 20-day precision (CLSI EP5-A3), reagent opened onboard stability (CLSI 25-A), interferences (CLSI EP7-A2), and cross-reactivity were also determined.

Results: For the Herpes-I IgG assay, PPA% was 99.6% and 99.0% and NPA% was 99.9% and 99.5%. The results of this study show that the prototype ADVIA Centaur Herpes-I IgG and Herpes-II IgG assays are highly specific immunoassays capable of determining HSV-1 IgG and HSV-2 IgG antibodies quantitatively in comparison to other assays. The robustness of the ADVIA Centaur Herpes-I IgG and Herpes-II IgG assays, together with the features of the ADVIA Centaur analyzers (random access, easy to use, and full automation), demonstrate their potential use in a laboratory setting.

Conclusions: The results of this study show that the prototype ADVIA Centaur Herpes-I IgG and Herpes-II IgG assays are rapid, precise, highly specific immunoassays capable of determining HSV-1 IgG and HSV-2 IgG antibodies quantitatively in comparison to other assays. The robustness of the ADVIA Centaur Herpes-I IgG and Herpes-II IgG assays, together with the features of the ADVIA Centaur analyzers (random access, easy to use, and full automation), demonstrate their potential use in a laboratory setting.

*Not for sale or distribution in the U.S.; not available in all markets.

Enabling Random Access Molecular Testing Using Multiple PCR Cells

D. Yang1, J. Fung1, N. Mueller2, G. Maurer2, Beckman Coulter Inc., Brea, CA, *Beckman Coulter Inc., Chaska, MN

OBJECTIVE: Beckman Coulter’s DxN VERIS Molecular Diagnostic System is a fully automated, random-access, sample-to-answer system for the quantitative/qualitative analysis of molecular targets. DxN VERIS incorporates the extraction, purification, quantification, and results interpretation of infectious disease nucleic acid targets using the real-time polymerase chain reaction (RT-PCR). The initial VERIS assay menu includes quantitative detection of Cytomegalovirus (CMV), Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Human Immunodeficiency virus Type 1 (HIV-1). The objective of this study was to test and report performance of each assay in limit of detection, clinical specificity, and method comparison.

Methods: Study methods were based on Clinical and Laboratory Standards Institute (CLSI) guidelines for “Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures” (EP17-A2E) and “Measurement Procedure Comparison and Bias Estimation Using Patient Samples” (EP9-A2). For clinical specificity assessed with the Wilson Score method, at least 287 samples were collected for each assay.

Results: Probit analysis calculated the DxN VERIS CMV Assay LoD as 15 IU/mL, supporting the product claim of 30 IU/mL, the DxN VERIS HBV Assay LoD as 3.8 IU/mL, supporting the product claim of 10 IU/mL, the DxN VERIS HCV Assay LoD as 4.3 IU/mL, supporting the product claim of 12 IU/mL, the 1 ml sample volume DxN VERIS HIV-1 Assay LoD as 30 IU/mL, supporting the product claim of 60 IU/mL, and the 0.175 ml sample volume DxN VERIS HIV-1 Assay LoD as 167 IU/mL, supporting the product claim of 400 IU/mL.

The average biases between VERIS assays and Roche COBAS assays were 0.24 log IU/mL for CMV, -0.32 log IU/mL for HBV, 0.20 log IU/mL for HCV, and 0.34 log IU/mL for HIV-1.

The Wilson Score method yielded 100% specificity with a lower bound confidence interval of 99.7% for CMV, 100% specificity with a lower bound confidence interval of 99.5% for HBV, 100% specificity with a lower bound confidence interval of 99.0% for HCV, and 99.8% specificity with a lower bound confidence interval of 99.1% for HIV-1 at the 1 ml sample volume assay.

Conclusions: Based on these data, the Beckman Coulter DxN VERIS CMV, HBV, HCV, and HIV-1 Assays are automated molecular tests with the sensitivity and accuracy of viral load monitoring required for effective patient management.

* The DxN VERIS System and DxN VERIS CMV, HBV, HCV, and HIV-1 Assays are not available for sale or use in the U.S.
Infectious Disease

Wednesday, August 3, 9:30 am – 5:00 pm

To compare the reproducibility across PCR cells, a pooled sample containing PCR-enabling reagents and two different genomic DNA targets was distributed among 20 PCR vessels, capped and loaded onto all 20 PCR cells. The samples were thermocycled using the same protocol and the data analyzed to obtain cycle threshold (Ct) values.

RESULTS: The temperature variability across all 20 cells during the 25 second dwell times was found to be less than 0.3°C in all cases. The largest variation, at 0.3°C, was measured at 95°C, while the smallest variation, at -0.1°C, was measured at 45°C. Amplification and real time detection of the two genomic targets resulted in Ct values of (32.46±0.15) and (28.40±0.16) cycles.

CONCLUSIONS: In order to meet throughput and single sample, random-access testing goals, the DxN VERIS Molecular Diagnostics System must contain PCR cells which all yield the same result for a given thermocycling protocol, irrespective of which cell is employed. This work demonstrated that the maximum PCR cell-to-cell temperature variation was less than 0.35°C, ensuring samples undergo comparable thermal experiences during the amplification and detection process. Actual real time PCR testing, using pooled samples, showed that the variance among the 20 cells tested was ≤ 0.16 cycles, indicating a high level of reproducibility.

*Not for sale or distribution in the U.S.; not available in all markets.

B-079

Lumipulse G TP-N Assay for Quantification of Anti-TP antibodies in Human Serum and Plasma

N. Benin1, S. Gannon1, C. Feldman1, J. Le2, K. Falcone3, S. Raju4, S. Jones1, C. Peacock1, N. White1, E. Eklund5, J. Hunsaker6, C. Lu1, J. Grenzen1, G. Lambert-Messerlian7, D. Grenache7, R. Radwan6, J. Young6, D. Dickson1,1 ‘Fujirebio Diagnostics, Inc., Malvern, PA, 2‘Women & Infants Hospital of Rhode Island, Providence, RI, 3‘Associated Regional and University of Pathologists Laboratories, Salt Lake City, UT

Background: Treponema pallidum (TP) is a pathogenic bacterium and the causative agent of syphilis. Assays for anti-TP have been adopted in clinical diagnostic testing, and are considered to be useful in the diagnosis of patients where infection with TP is suspected, or in preventing infection via blood transfusion (Larsen, 1995; Singh, 1999; LaFond, 2006 and Meyer, 1996). Lumipulse G TP-N can be used as an initial screening test or in conjunction with a non treponemal laboratory test and clinical findings to aid in the diagnosis of syphilis infection. WARNING: Lumipulse G TP-N is not intended for blood and tissue donor screening. United States federal law restricts this device to sale by or on the order of this physician.

Methods: Lumipulse G TP-N is an immunnoassay for the qualitative detection of anti-TP antibodies in serum or plasma based on CLEIA technology by a two-step sandwich immunnoassay method on the LUMIPULSE G System. In the assay, serum or plasma was incubated with recombinant TP antigen-linked magnetic particles to capture the analyte, anti-TP antibodies. The particles were then washed and rinsed to remove unbound materials. Alkaline phosphatase-labeled recombinant TP antigens were added to and incubated with the anti-TP antibodies-bound particles. The particles were then washed and rinsed again to remove unbound materials. Substrate 3-(2’-spiroadamantane)-4-methoxy-4-(3”-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt (AMPPD) solution was then added to and mixed with the particles. Luminescence signals were thus generated by the cleavage reaction of dephosphorylated AMPPD and converted into the amount of anti-TP antibodies in the serum or plasma.

RESULTS: The Lumipulse G TP-N demonstrated a 20-days total imprecision ≤ 5.3% (n = 80), site to site ≤ 5.4% (n = 120) and Lot to lot ≤ 6.7% (n =120) using 5 serum, one Na Citrate plasma panel and one commercially available control. One thousand seven hundred forty-five (1745) samples were used to compare the Lumipulse G TP-N with the Final Comparator Result using a Treponemal IgG/IgM test (Centaur ADVIA), Nontreponemal test (RPR), and 2* Treponemal test (Serodia TP-PA). The Positive Predictive Agreement (PPA) was 96.8% and the Negative Predictive Agreement (NPA) was 98.0%. The PPA results of Lumipulse G TP-N for a total of 724 apparently healthy patients (apparently healthy adults, children and pregnant women) were 99.7%. Two samples tested positive (1 apparently healthy adult and 1 pregnant woman) for Lumipulse G TP-N. The apparently healthy adult was confirmed positive by Serodia TP-PA. The pregnant woman was negative per Serodia TP-PA.

The Lumipulse G TP-N demonstrated an average interference of ≤ 10% (for each compound) in a study with human serum specimens supplemented with potentially interfering compounds individually at indicated concentrations: 1000 ng/mL HAMA, 1000 IU/mL rheumatoid factor, 40 mg/dL conjugated and free bilirubin, 3000 mg/dL triglycerides, 500 mg/dL hemoglobin, 20 g/dL protein, 500 ng/mL glucuronic acid 50mg/dL, and ascorbic acid 3.5mg/dL.

Conclusion: Lumipulse G TP-N assay under development demonstrated to be a precise, sensitive, specific and robust assay for the quantitative determination of anti-TP antibodies in human serum and plasma.

B-080

Evaluation Of Measles IgM & IgG Seroprevalence From Ankara 2nd Region Central Laboratory

Y. Buzkaya1, G. Aksu1, E. Kas1, K. Mintas1, S. Çopur1, C. Atay Sonmez, K. murat1, F. Guelc1, B. Kara1, N. Aksel Bayram. Public Hospital Administration of Turkey Ankara 2nd Region Central Laboratory, Ankara, Turkey

Background: Measles is a common, infectious disease of childhood. The addition of measles vaccine to the routine vaccination programme has fewered the number of

S166 68th AACC Annual Scientific Meeting Abstracts, 2016
Development of a Rapid Point-of-Care Molecular Assay for Diagnosis of Influenza and RSV


Background: Rapid, accurate differential diagnosis of influenza is important for selective effective treatment. Current CLIA-waived rapid immunodiagnostic tests for influenza target proteins on the virus surface and, due to the rapidly mutating nature of this virus, may lack adequate sensitivity and/or specificity in any given flu season. Newer CLIA-waived molecular diagnostics targeting conserved regions of the viral RNA have better and more consistent sensitivity and specificity, but require expensive, dedicated instruments and cold storage of assay reagents, limiting their utility in outpatient settings.

Methods: Mesa Biotech has developed a self-contained molecular diagnostic platform specifically designed for CLIA waiver and ease of use in the physician’s office outpatient setting. The small, inexpensive, reusable deck and disposable assay cassettes combine to form a user-friendly system. The assay cassette integrates nucleic acid extraction, nucleic acid amplification, and lateral flow-based colorimetric hybridization detection into one easily used device that can be stored at room temperature. Within ~30 minutes of collecting a nasal swab sample, the user can obtain a laboratory-quality molecular influenza test result and take the appropriate therapeutic action.

Results: A pre-clinical study run at three US physician office sites in January-March 2015 demonstrated the assay protocol was simple for physician office staff to master and Flu A/Flu B assay data was obtained on the first attempt for 186 patient samples. In this setting the Mesa Biotech cassette showed good sensitivity for Flu A when compared to the Focus Simplexa Flu A/B & RSV assay (86%) and viral culture (88%), but showed inadequate sensitivity for Flu B. Specificity of both assays was ~99% in this pre-clinical study. The test cassette has since been further developed to improve the sensitivity for both Flu A and Flu B and to add detection of respiratory syncytial virus (RSV). In-house studies on a panel of influenza and RSV strains and isolates has shown good strain coverage for influenza A, influenza B, and RSV A/B. Testing for specificity with a panel of frequently encountered non-influenza nasal microorganisms indicates no cross reactivity.

Conclusions: A clinical study is in current progress at ten geographically distributed US sites to evaluate the performance of this improved assay to detect and differentiate influenza A, influenza B, and RSV. Two nasal swabs are being collected from patients with signs and symptoms of influenza-like illness. One swab is immediately placed into the Mesa Biotech assay sample buffer and frozen for later analysis. The second nasal swab is placed into viral transport medium and transported to a central laboratory for analysis with the Focus Simplexa Flu A/B & RSV test. After clinical sample collection is completed in April/May 2016, the blinded Mesa Biotech samples will be thawed, run with the Flu A/Flu B/RSV cassette, read visually, and results recorded. To date over 450 clinical nasal swab samples have been collected with a positivity rate by the Simplexa assay of 10.7% for Flu A, 11.1% for Flu B, and 16.8% for RSV. Final results from the completed 2016 clinical study of the improved Mesa Biotech Flu A/Flu B/RSV assay will be presented.
Evaluation of the Architect Syphilis assay after reformulation

E. Sickinger1, M. Anderson2, V. Salibilla1, M. Hoffmann1, U. Spies1, C. Kochl2, S. Hershberger2, L. Gu2, G. Williams1, G. Schlauder1, 2Abbott GmbH & Co KG, Wiesbaden-Delkenheim, Germany; 2Abbott Diagnostics, Abbott Park, Chicago, IL

Background: Testing for Syphilis specific antibodies is recommended for pregnant women to prevent congenital infections and members of at risk populations such as MSM or HIV infected patients to prevent further spread of the disease, and is a mandatory test for blood donations in many countries. As such, any Syphilis test has to be highly specific to reduce the number of false reactive results to a minimum. In response to this need the ARCHITECT Syphilis TP assay was reformulated with the aim of improved precision and specificity.

Methods: Evaluation of the new version of ARCHITECT Syphilis TP assay included testing of 5180 random blood donor specimens (serum and plasma) for determination of specificity and 409 samples pre-characterized as reactive for Syphilis antibodies (by various test methods) to determine sensitivity in comparison to the previous assay version. Samples with reactive results were further tested by 3 immunoblots: INNO-LIA™ Syphilis Score, Mikrogen recomLine Treponema IgG and recomLine Treponema IgM for confirmation. A sample was defined as positive if reactive in at least two of 4 assays, including the previous ARCHITECT Syphilis assay version. Imprecision was determined according to protocol EP5-A2 using the assay specific negative and positive controls and 4 plasma panels.

Results: Resolved specificity of the new assay version was 99.94 % (5171/5174). One sample was defined positive and 5 samples were unable to categorize and excluded from calculation of specificity. All samples pre-characterized as containing Syphilis antibodies were found reactive (409/409). Within-laboratory imprecision was determined to be 3.6 % CV for the Positive Control and ranging from 2.0 - 2.9 % for the 4 plasma panels.

Conclusion: The new version of the ARCHITECT Syphilis TP assay showed improved specificity compared to the previous version without compromising sensitivity. Lot to lot variation was reduced demonstrated by an imprecision of less than 4 %.

Zika virus in Brazil: a prevalence study

L. B. Alvim1, O. S. D. Roman1, E. Mateo2, A. C. S. Ferreira2, D. A. G. Zauli1, 1Hermes Pardini Institute (Molecular Genetics Sector), Vespasiano, Brazil, 2Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil

Background: Zika virus (ZIKV) is an RNA virus of family Flaviviridae and genus Flavivirus transmitted by mosquitoes of genus Aedes, including specie Aedes aegypti. It was initially isolated in 1947 in the Zika forest in Uganda, and nonspecific clinical features of Zika fever (FZ) can be confused with most other arboviruses particularly dengue and chikungunya virus infection. The first ever case of ZIKV disease in Brazil was reported in May 2015 and since then the virus has rapidly spread within Brazil and across 22 other countries. Currently, 534 out of 72,062 Zika suspected cases reported in Brazil were confirmed. The outbreaks suggests that FZ is an emerging disease and its might be associated with the increasing numbers of congenital microcephaly cases reported in the country. Therefor, there is an urgent need to detect and study the prevalence of Zika virus in Brazil and regions in order to contribute to the implementation of public health policies in Brazil. Objective: To report the prevalence of Zika virus infection in Brazil and federative units during period of December 2015 to February 2016. Methods: The detection of ZIKV was performed by RT-qPCR with specific primers and TaqMan fluorescent probe technology. The inclusion criterion was the detection of viral RNA in patients. Results: A total of 585 patients were analyzed, among these 137 (23.4%) from Midwest region, 145 (24.8%) from Northeast region, 42 (7.2%) from North region, 230 (39.3%) from Southeast region, 31 (5.3%) from South region. Only one state (Acre) has not been studied. It was observed that the median age of patients was 35 years, and most patients were female (73.8%). Among the samples evaluated 12.14% (71/585) were ZIKV positive. The Midwest region presented 19.7% (27/137) of positive cases, North region 19.5% (8/42), Southeast region 12.2% (28/230), Northeast region 4.8% (7/145) and South region 3.2% (1/31). The data showed that state of Bahia had 43% of positive results of Northeast region; Minas Gerais state had 75% of Southeast region. Goias state had 52% of Midwest region and Tocantins had 50% of North region.

Conclusion: The results obtained by ZIKV specific molecular testshowed that Midwest region has the highest prevalence of zika virus infection followed by North region and South region. However, the published data indicate that the Northeast is the most affected. This prevalence study of ZIKV infection in different regions of Brazil may lead to a clearer indication/virus potential to spread and establish in the country. Further investigations are needed to improve our understanding the disease and its clinical effects such as possible connections between the virus and neurologic complications.

Cross-Sectional Study of Patients Tested for 5 Sexually Transmitted Infections Using Molecular Methods

O. S. D. Romano1, F. L. O. Martinho1, E. Mateo2, D. A. G. Zauli1, 1Hermes Pardini Institute (Molecular Genetics Sector), Vespasiano, Brazil, 2Hermes Pardini Institute, Vespasiano, Brazil, 3Hermes Pardini Institute (Research and Development Sector), Vespasiano, Brazil

Background: According to the World Health Organization more than 1 million sexually transmitted infections (STIs) are acquired every day worldwide. There are more than 30 different species of bacteria, viruses, and parasites that are sexually transmitted, and several of these agents can be transmitted during pregnancy and/or shortly after birth. STIs often involve more than one pathogen, difficulting the diagnosis and increasing the treatment cost, besides increase susceptibility to Human Immuno deficiency Virus (HIV) infection. Neisseria gonorrhoeae and Chlamydia trachomatis cause major causes of pelvic inflammatory disease and infertility in women. Mycoplasma genitalium is associated with non-gonococcal urethritis, pelvic inflammatory disease, spontaneous abortions and infertility. It is often present concurrently with Ureaplasma species. Ureaplasma urealyticum is related with urogenital tract infections, abotions and infertility. Objective: To investigate the prevalence of Mycoplasma hominis, Mycoplasma genitalium, Ureaplasma urealyticum, Neisseria gonorrhoeae and Chlamydia trachomatis among patients tested for STIs. Method: This is a cross-sectional study of data from patients who were tested for STIs in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) in the period of 2010 to 2012. The inclusion criteria was the detection of DNA of one or more microorganisms by using polymerase chain reaction. Results: From a total of 2,332 samples analyzed, 1,393 (59.7%) were positive for one or more microorganisms, and 939 (40.2%) were negative. 1,334 (57.2%) patients were male and 998 (42.7%) female. Mycoplasma hominis was the most prevalent microorganism among both sexes, 509 (78.5%) men and 486 (71.2%) women. Overall, men had a higher positivity rate than woman, with 711 (51.0%) versus 682 (48.9%) for STIs. Among the positive samples, 1,160 (83.2%) were detected a single microorganism, and 233 (16.7%) were detected multiple microorganisms. 995 (71.4%) samples were positive for STIs. Among the positive samples, 1,160 (83.2%) were detected a single microorganism, and 233 (16.7%) were detected multiple microorganisms. 995 (71.4%) samples were positive for STIs. Among the positive samples, 1,160 (83.2%) were detected a single microorganism.
**Infectious Disease**

**B-088**

**Performance of the HIV Ag/Ab Combo and Syphilis assays on Abbott’s next-generation immunochemistry analyzer**

E. Sickinger, H. Braun, B. Heinrich, C. Buemming, Abbott GmbH & Co KG, Wiesbaden-Delkenheim, Germany

**Background:** HIV Ag/Ab Combo assays are able to detect HIV p24 antigen and anti-HIV antibodies simultaneously, therefore reducing the risk of transfusion transmitted HIV compared to assays detecting HIV antibodies exclusively. Syphilis assays are intended to be used as an aid in the diagnosis of Syphilis infection and as a screening test to prevent transmission of Treponema pallidum to recipients of blood, blood components, cells, tissue and organs. The aim of the current study is to evaluate the key performance characteristics like sensitivity and specificity of the HIV Ag/Ab Combo and Syphilis assays on a next-generation immunochemistry analyzer in comparison to the on-market comparator analyzer.

**Methods:** The HIV Ag/Ab Combo and the Syphilis assays were evaluated internally in regards to specificity and sensitivity. For HIV Ag/Ab Combo and Syphilis results were generated on the next-generation immunochemistry analyzer (test to be evaluated= TBE) and compared to results generated on the on-market comparator analyzer (test of record =TOR).

**Sensitivity**

**HIV Ag/Ab Combo**

Antigen Sensitivity: The analytical HIV-1 p24 antigen sensitivity was determined by evaluation of the WHO International Standard HIV-1 P24 Antigen NIBSC (code: 90/636) and the BIO-RAD HIV-1 Antigen Standard (LN 72217). Antibody Sensitivity: Clinical sensitivity was evaluated on selected HIV-1 (n = 430 including 43 HIV-1 g0 specimens) and HIV-2 (n = 115), positive specimens. Seroconversion sensitivity was assessed on sequential specimens from 37 seroconverting donors.

**Syphilis**

Sensitivity of the Syphilis assay was evaluated on the WHO 05/122 and WHO 05/132 standards and on 415 confirmed positive diagnostic specimens.

**Specificity**

Specificity of the HIV Ag/Ab Combo assay was investigated on 5340 blood donor specimen and on 211 diagnostic specimens. Specificity of the Syphilis assay was investigated on 5119 blood donor specimen and on 531 diagnostic specimens.

**Results:**

Antigen sensitivity determined per linear regression ranged from 20.41 - 20.81 pg/mL for the TBE and was at 21.01 pg/mL for the TOR using the Bioserad HIV Antigen Standard. The same assays exhibited antigen sensitivities of 0.73 - 0.75 IU/mL and 0.77 IU/mL using the WHO Standard. Antibody sensitivity was at 100% on both platforms. The first reactive time point for the TBE HIV Ag/Ab Combo assay occurred earlier in 2 panel sets and as the same time in the 35 remaining panel sets compared to the TOR. The HIV Ag/Ab Combo assay TBE exhibited a specificity of 99.93% (5336/5340) compared to 99.91% (5364/5369) on the TOR. The diagnostic specificity was at 99.53% (210/211) for both platforms.

The Syphilis TBE showed a clinical specificity of 99.52% (413/415). The two missed specimens were also negative on TOR. Donor specificity was at 99.94% (5116/5119), clinical specificity at 100.00% (531/531) on the TBE and 99.76% on the TOR. Analytical sensitivity was 0.01 IU/mL as measured against both WHO standards: 05/122 and 05/132.

**Conclusion:** The HIV Ag/Ab Combo and Syphilis assays showed equivalent or better performance in terms of sensitivity and specificity on the next-generation immunochemistry analyzer compared to the on-market comparator analyzer.

---

**B-089**

**Concordance for HIV Combo assay between Chemiluminescence and Electrochemiluminescence platforms**

M. F. Mantovani, O. Fernandes, O. Osório, M. C. Trentin. DASA - Diagnósticos da América, São Paulo, Brazil

**Objective:** Laboratories face a challenge regarding the CMV assays, mainly for the IgM tests. One of the principal issues to be solved is the IgM residual positive results. Some methodologies are more sensible and variations between positive and negative results for the same patient aren’t rare. This study aims to compare concordance of CMV assays between the platforms Cobas E-170 (Roche) and ADVIA CentaurXP (Siemens Diagnostics) in a large laboratory in Cascavel, Brazil.

**Materials and Methods:** 641 and 578 samples were selected from the routine to be processed in the CMV IgM and IgG respectively. Samples were tested in ADVIA Centaur and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies.

**Results and Discussion:** The results obtained in this study are showed in the Table 1.

---

**B-090**

**Concordance for CMV assay between chemiluminescence and electrochemiluminescence platforms**

M. F. Mantovani, O. Osório, O. Fernandes, L. G. S. Carvalho. DASA - Diagnósticos da América, São Paulo, Brazil

**Objective:** Laboratories face a challenge regarding the CMV assays, mainly for the IgM tests. One of the principal issues to be solved is the IgM residual positive results. Some methodologies are more sensible and variations between positive and negative results for the same patient aren’t rare. This study aims to compare concordance of CMV assays between the platforms Cobas E-170 (Roche) and ADVIA CentaurXP (Siemens Diagnostics) in a large laboratory in Cascavel, Brazil.

**Materials and Methods:** 641 and 578 samples were selected from the routine to be processed in the CMV IgM and IgG respectively. Samples were tested in ADVIA Centaur and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both. The discordant results (initially reactive for one of the tests) were processed in an Avidity IgG assay in an attempt to determine the result of better specificity.

**Results and Discussion:** The results are showed in the Table 1:

---

**Table 1:** Results of comparative analysis.

**Conclusion:** The results showed a good correlation (97.27%) between the eHIV assays analyzed, considering only the first result for each assay (initially reactive). The differences (2.73%) in the results of eHIV between platforms Siemens and Roche can be clarified by duplicate repetition, confirmatory assay WB and third assay with similar methodology.
Concordance for HVA Total and IgM assay between chemiluminescence and electrochemiluminescence platforms

M. F. Mantovani, O. Fernandes, P. Osório, D. C. Hamerski. DASA - Diagnósticos da América, São Paulo, Brazil

Objective: The assays for HAV Total and HAV IgM are used to define the serological status for Hepatitis A. Some assays have different specificities and sensitivities and the laboratories deal with some variant results. The present study aims to compare concordance of HAV Total and HAV IgM assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics), in a large laboratory in Cascavel, Brazil.

Materials and Methods: 279 and 408 samples were selected from the laboratory routine to be tested in HAV Total and HAV IgM, respectively. Samples were tested in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. Reactive samples were used to the precision test. The correlation was performed based in the first result without a duplicate repetition in both methodologies. High and low reactive and samples were used to the precision test in 2 different sample pools repeated 20 times.

Results and Discussion: The results concerning the present study are showed in the Table 1.

Table 1: Comparative results.

<table>
<thead>
<tr>
<th>Total Samples</th>
<th>HAV Total</th>
<th>Reactive Samples Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant Results</td>
<td>268</td>
<td>96.06%</td>
</tr>
<tr>
<td>Nonreactive Samples Agreement</td>
<td>73</td>
<td>26.16%</td>
</tr>
<tr>
<td>Reactive Samples Agreement</td>
<td>195</td>
<td>68.89%</td>
</tr>
</tbody>
</table>

The HAV Total assay showed 4.30 % for within run precision and 6.57% for between runs precision. The HAV IgM assay within run precision was 6.13% and 4.08% for the between runs precision.

Conclusion: The results showed a good correlation (96.06% and 99.26%) between the HAV Total and HAV IgM assays analyzed, considering only the first result for each assay (initially reactive). The differences in the results of HAV Total (3.94%) and HAV IgM (0.74%) between platforms Siemens and Roche can be clarified by duplicate repetition, third assay with similar methodology and patient historic.

Concordance for HBV markers assay between chemiluminescence and electrochemiluminescence platforms

M. F. Mantovani, O. Fernandes, P. Mendonça, P. Osório. DASA - Diagnósticos da América, São Paulo, Brazil

Objective: The laboratory labors with different performance to the screenings assays, mainly with the HBV markers. There are some false reactive results due different specificities for the assays. The laboratories are facing a challenge to diagnostic HBV with good performance assays. The present study aims to compare concordance of HBV assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics), in a large laboratory in Cascavel, Brazil.

Methods: 6526 samples were selected from the laboratory routine to be tested in the HBV markers. Samples were tested in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies. Reactive samples were used to the precision test in a pool sample repeated 20 times.

Results: The results from this study are demonstrated in the Tables 1 and 2:

<table>
<thead>
<tr>
<th>Assay (Total Samples)</th>
<th>HBAg (2479)</th>
<th>Anti-HBs (2258)</th>
<th>HBeAg (517)</th>
<th>HBC IgM (432)</th>
<th>Anti-HBc (441)</th>
<th>HBeAg (405)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant Sample% (n)</td>
<td>97.9 (2420)</td>
<td>96.1 (2170)</td>
<td>97.5 (504)</td>
<td>98.1 (424)</td>
<td>94.3 (416)</td>
<td>99.0 (401)</td>
</tr>
<tr>
<td>Discordant Sample% (n)</td>
<td>0.9 (21)</td>
<td>1.3 (30)</td>
<td>2.5 (13)</td>
<td>0.5 (2)</td>
<td>1.0 (4)</td>
<td>0.6 (21)</td>
</tr>
</tbody>
</table>

Table 1: Comparative results.

The results showed a good correlation between the HBV markers assays analyzed, considering only the first result for each assay (initially reactive): HBSAg 97.86%, anti-HBs 96.10%, anti-HBe Total 97.49%, anti-HBc IgM 98.15%, anti-HBc IgM 94.33% and HBeAg 99.01%. The differences in the results of HVB between platforms Siemens and Roche can be clarified by duplicate repetition and also third assay with similar methodology.

Evaluation of modified FibroTest Formula in Patients with Genotype 4 Hepatitis C

W. Tamimi, R. M. AlDarrab, N. Alotabi, A. Aljumah, S. Almaman, I. Altriaif. King Abdullah International Medical Research Center & King Salub Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

Background: Liver fibrosis is usually diagnosed with core needle liver biopsy which is considered to be an invasive procedure and may be harmful to the patient. Non-invasive methods and formulas have been suggested to replace these invasive procedure. For example, “FibroTest” formula has been validated for the initial diagnosis of fibrosis and for the monitoring of patients. The “Fibrotest” formula is based on the calculations of blood biochemical parameters. Many of these formulas where only validated in developed countries with HCV genotype 1 patients. Conversely, limited studies have been conducted in the developing countries including Saudi Arabia, where genotype 4 predominates. The main objective of this study is to validate and evaluate some the non-invasive modified “Fibrotest” formulas in hepatitis C genotype 4 patients with or without liver fibrosis. Methods: A cross sectional study that utilized adult genotype 4 HCV patients who attend the hepatology clinic. Two groups were initially evaluated for fibrosis using transient elastography “FibroScan”, “Fibrotest” and liver biopsy. Metavir histological scoring system were used for staging of fibrosis in liver biopsies. The “Fibrotest” formula which includes 6 serum markers, Alpha-2-macroglobulin, Haptoglobin, ApolipoproteinA1 (Apo A1), Gamma-glutamyl transpeptidase (GGT), Total bilirubin and Alanine transaminase (ALT). Along with patients’ age and gender. The modified fibrotest formula was used by replacing the Apo A1 with high density lipoprotein (HDL). All of these markers were measured in serum by Architect analyzer from Abbott. The first group included 26 patients diagnosed with hepatitis C genotype 4 with mild fibrosis (F0, F1) on fibrotest. The second group included 54 hepatitis C patients moderate and severe liver fibrosis (F2, F3, F4) on fibrotest. Fibrotest and modified fibrotests were calculated using markers and compared with the biopsy and fibroscan in both groups. Results: The samples consisted of 46 Females (57%) and 34 males (43%) with a mean age of 53 years (sd 12). When both fibrotest and the modified fibrotest were compared, agreement was found to be 80% in stage F0, 72% in F1, 67% in F2, 50% in F3 and 96% in F4. When fibrotest was compared with biopsy, agreement was found in 58% of F0, 78% of F1, 18% of F2 and 35% of F4. Similar results were found with the modified fibrotest by 67% in F0, 78% in F1, 15% in F2, and 32% in F4. Finally, comparison of fibrotest and fibroscan were found to be with agreement in 50% of F0, 100% of F1, 10% of F2, 60% of F3 and 54% of F4. Modified fibrotest and fibroscan was agreed in 50% of F0, 100% of F1, 14% of F2, 50% of F3 and 47% of F4. Conclusion: Both fibrotest and the modified fibrotest were comparable to each other and moderately to fibroscan. However, the use of noninvasive fibrosis formulas were shown to be less superior to liver biopsy in our study especially at late stage of liver fibrosis.
Concordance for Rubella IgM and IgG assay between chemiluminescence and electrochemiluminescence platforms

M. F. Mantovani, O. Fernandes, P. Osório, L. G. S. Carvalho. DASA - Diagnósticos da América, São Paulo, Brazil

Objective: Laboratories regularly find out difficulties in the Rubella assays, mainly for the IgM tests. IgM residual results in IgG positive patients and reactions with unspecific antibodies are some issues to be solved. Some methodologies are more sensible and variations between positive and negative results for the same patient aren’t rare. This study aims to compare concordance of Rubella IgM and IgG assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics) in a large laboratory in Cascavel, Brazil.

Materials and Methods: 497 and 471 samples were selected from the laboratory routine to be processed in the Rubella IgM and IgG respectively. Samples were tested in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies.

Results and Discussion: The results obtained in this study are showed in the Table 1:

<table>
<thead>
<tr>
<th>Total Samples</th>
<th>Rubella IgM</th>
<th>Rubella IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concordant Results</td>
<td>402</td>
<td>80.56%</td>
</tr>
<tr>
<td>Nonreactive Samples Agreement</td>
<td>373</td>
<td>74.75%</td>
</tr>
<tr>
<td>Reactive Samples Agreement</td>
<td>26</td>
<td>5.21%</td>
</tr>
<tr>
<td>Inconclusive Samples Agreement</td>
<td>3</td>
<td>0.60%</td>
</tr>
<tr>
<td>Discordant Results</td>
<td>95</td>
<td>19.04%</td>
</tr>
<tr>
<td>Nonreactive Siemens x Reactive Roche</td>
<td>60</td>
<td>12.02%</td>
</tr>
<tr>
<td>Nonreactive Siemens x Inconclusive Roche</td>
<td>24</td>
<td>4.81%</td>
</tr>
<tr>
<td>Reactive Siemens x Inconclusive Roche</td>
<td>9</td>
<td>1.80%</td>
</tr>
<tr>
<td>Reactive Siemens x Inconclusive Roche</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inconclusive Siemens x Reactive Roche</td>
<td>2</td>
<td>0.40%</td>
</tr>
<tr>
<td>Inconclusive Siemens x Inconclusive Roche</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Total Samples Results Classification

Conclusion: The results showed similar correlations for Rubella IgM (89.94%) and IgG assay (80.56%). The differences in the results of Rubella IgM are due the IgM residual results, when both IgM and IgG are reactive for the assays. Those differences between platforms Siemens and Roche can be clarified in routine using a third assay and also the Avidity IgG Assay.

Pre-clinical performance of the FluChip-8G assay, a high information content in vitro diagnostic assay


Background: The current paradigm in molecular diagnostics for respiratory illness typically involves assays based on real-time RT-PCR or microarrays to detect small portions of a pathogen’s genome as an indicator of presence/absence of a target pathogen. InDevR’s FluChip-8G Influenza A+B Assay is a high information content molecular diagnostic assay that provides same day identification and differentiation of influenza viruses detected in clinical samples. Multiple whole gene segments of influenza A and B are RT-PCR amplified, fragmented, and hybridized to a DNA microarray containing over 450 unique capture sequences representing a large fraction of the influenza genome (the microarray represents ~72% and ~88% gene portions of a pathogen's genome as an indicator of presence/absence of a target pathogen). Automated data analysis on the microarray images is performed using a pattern recognition-based mathematical algorithm. The FluChip-8G assay provides clinically-relevant information including influenza typing (A or B), seasonal A virus subtyping (H1N1pdm2009 and H3N2), linear performance characteristics for influenza B viruses, and the unique ability to positively identify the presence of “non-seasonal” influenza A viruses. Importantly, the assay conducted on clinical samples can also provide information for epidemiological/public health/research purposes, including identification of potentially pandemic non-seasonal influenza A viruses (differential between a variety of relevant subtypes), and presence/absence of the H275Y and E119V NA mutations (putative antiviral resistance markers). Method: The objectives of this study were to investigate pre-clinical performance characteristics for the FluChip-8G assay; perform pre-clinical study execution. Performance of the FluChip-8G assay was compared to a CDC panel of FDA-cleared influenza diagnostic assays on a set of 119 retrospective clinical nasopharyngeal swab specimens collected between 2007-2015. In addition, cross-reactivity and interference was investigated by analyzing the pathogens and substances recommended in relevant FDA guidance documents. Results: For the 119 specimens tested, negative percent agreement (NPA) between the two assays was 100% for all target analytes, indicating no false positive results for the FluChip-8G assay. Positive percent agreement was 96% (24/25) for A/H1N1pdm(2009), 91% (32/35) for A/H3N2, 92% (12/13) for B/Yamagata, 100% (14/14) for B/Victoria, and 100% (13/13) for “non-seasonal” Flu A (known to be pre-2009 A/H1N1). In addition, no cross-reactivity with the FDA-recommended panel of potentially cross-reactive organisms was observed, and the only substance resulting in assay interference was the FluMist® live influenza vaccine. Conclusion: Pre-clinical studies performed on the FluChip-8G assay indicate comparable performance to a suite of FDA-cleared CDC singleplex real-time RT-PCR based diagnostic assays and no cross-reactivity with other common respiratory tract organisms. Importantly, 3 pre-2009 H1N1 viruses (no longer the currently circulating seasonal strain) were correctly called “non-seasonal” influenza A by the FluChip-8G Assay. Prospective, multi-site clinical studies are planned for initiation in Q2 of 2016.

Evaluation of Novel Respiratory Assays on the Fully Automated Panther Fusion System


Background: Respiratory viral infections remain a leading cause of infectious diseases worldwide. Since most respiratory viruses present with similar symptoms and many pathologies are co-infected, clinicians rely on molecular diagnostic methods to quickly and accurately identify one or more viral agents to ensure appropriate patient management. Multiple novel respiratory assays are being developed to run on the fully automated Panther Fusion system (in development) with random access capability, minimum sample handling, and short load-to-first result. The Panther Fusion Flu A/B/RSV, Para 1/2/3/4, and Adv/MPMV/RV assays can all be run from a single nucleic acid isolate. These qualitative multiplex RT-PCR assays detect multiple targets utilizing individualized assay specific reaction pellets. Performance of the Panther Fusion Flu A/B/RSV assay which detects and differentiates Flu A, Flu B, and RSV, and the Panther Fusion Parafflu assay, which detects and differentiates human Parainfluenza (HPIV) types 1, 2, 3 and 4 were evaluated to demonstrate rapid, reproducible, and reliable detection of respiratory targets.

Methods: Reproducibility was tested at 0.5 log above LoD using virus spiked in nasopharyngeal clinical matrix and tested for both assays. Clinical performance of each viral target was compared to various on-market assays.

Results: Percent agreement of the reproducibility study for all intended targets for both assays was 100%. Concordance of clinical performance to-on-market assays was high with positive and negative agreements of 96.5-100% and 96.0-100%, respectively, for all intended targets for both assays. Time-to-first result was less than 2.5 hours.

Conclusion: These results demonstrate the Panther Fusion Respiratory Assays, Panther Fusion Flu A/B/RSV, and Parafflu assays are suitable candidates for repeatable and accurate identification of Influenza A, Influenza B, RSV, and HPIV subtypes 1-4.

Plural fluid lactate for diagnosis and management of parapneumonic pleural effusion

J. D. Santotoribio1, H. Alnayed-Hanwi2, P. Batalha-Caetano3, S. Pérez-Ramos1, M. J. Sanchez del Pino1,1. Puerto Real University Hospital, Cádiz, Spain, 2Cádiz University School of Medicine, Cádiz, Spain, 3Virgen del Rocio University Hospital, Sevilla, Spain

Background: Parapneumonic pleural effusions (PPE) are exudates associated to pneumonia, lung abscess or bronchiectasis, they are called empyemas when containing pus. Approximately 40% of patients hospitalized with pneumonia have pleural effusions. Morbidity and mortality are higher in patients with pneumonia and pleural effusion than in those without pleural effusion. In the management of PPE, the relevant challenge is to discriminate between uncomplicated (UPPE) and complicated PPE (CPPPE) for identify which PPE require chest tube drainage (CPPPE and empyemas). Pleural fluid lactate levels may be useful for diagnosis and management of PPE; however, there are very few published studies on the quantification of lactate in pleural fluid. New studies assessing the diagnostic value of pleural fluid lactate are necessary. The aim of this study was to measure the accuracy of pleural fluid lactate

68th AACC Annual Scientific Meeting Abstracts, 2016 S171
Total bile acid levels in hepatitis C RNA positive patients with liver fibrosis


Background: Bile acids (BA) are liver-specific products of cholesterol metabolism, and their measurement in blood is used as test of hepatobiliary dysfunction. Furthermore, BA have been associated with increased viral replication in hepatitis C virus (HCV) infected cells. BA levels were also shown to positively correlate with increasing stage of liver fibrosis in HCV patients. Our objective was to investigate the relationship between BA levels, HCV viral load, and liver fibrosis stage in order to assess the utility of total BA as an HCV monitoring test.

Methods: 172 patient samples (110 men, 62 women) tested for HCV viral load by real-time PCR were frozen at -20°C prior to BA testing. The total BA levels were quantified using Diazyme reagent on Beckman DxC instrument. All patients had fibrosis stage determined by FibroSure.

Results: Among the 172 patient samples, 84 (49%) had HCV RNA > 15 IU/mL, while 88 (51%) had HCV RNA < 15 IU/mL. Patients with fibrosis stages 1-4 in HCV RNA > 15 IU/mL group had higher mean total BA levels compared to HCV RNA < 15 IU/mL group, and they showed increasing BA levels consistent with fibrosis stage (Figure 1). However, the difference in BA levels between HCV RNA < 15 IU/mL and > 15 IU/mL groups was statistically significant only for patients with stage 4 fibrosis, with total BA means of 8.7 µmol/L (95% CI of 8.0 - 13.2) and 24.5 µmol/L (95% CI of 18.5 - 31.2), p < 0.001, respectively.

Conclusions: An increasing trend in BA levels relative to fibrosis stage in patients with HCV RNA > 15 IU/mL was observed. The statistically significant difference in BA levels among the two patient groups was confined to stage 4 fibrosis. Total BA test may be useful as a monitoring tool, particularly for patients with stage 4 HCV fibrosis.

Calprotectin evaluation as an inflammation marker in different stages of Kawasaki disease

M. A. A. L. Almeida, C. M. R. Magalhães, N. Selleski, L. Gandolfi, R. Pratesi, Y. K. M. Nóbrega. Universidade de Brasília, Brasília, Brazil

Kawasaki disease (KD) is the most common systemic vasculitis syndrome that primarily affects infants and young children. KD is in most cases a self-limited illness resolving within few weeks after fever onset. It preferentially affects small and medium-sized arteries, particularly the coronary arteries, and in 15 to 25% of patients, it will result in coronary aneurysms or dilatation. The natural history of coronary artery lesions in KD furnish evidences for a continuous active pathological process after the decline of acute symptoms. According to histological studies, even apparently normal coronary artery with no history of dilated lesions could show some degree of intimal thickening several years after the disease onset. Consequently, effective markers of inflammation and autoimmunity are needed for an effective follow-up of KD children both during the acute and subacute phase as during the following years.

Among several inflammation markers available, Calprotectin (CP), a calcium- and zinc-binding protein of the S100/calgranulin family has been successfully used as a marker in several inflammatory and autoimmune diseases. The objective of the present study is to evaluate the use of Calprotectin (CP) as a serological marker in the assessment of inflammation in patients during the different stages of KD. Thirty patients (2.5 years ± 1.2) were selected from January 2013 to December 2014, among patients referred to the Brasilia Children’s Hospital Jose Alencar (Federal District, Brazil) for KD diagnostic confirmation and follow-up. Serum samples were collected following standard H3-A6 of Clinical and Laboratory Standards Institute (CLSI) and stored at -80°C until analysis. Calprotectin was assayed by ELISA method using a commercial kit according to the manufacturer’s instructions. For analysis, the samples were stratified into three groups according to the stage of KD (acute, subacute and chronic) and calprotectin levels were detected at three different times: during the first 10 days of disease (acute phase, n = 3), between 30 and 60 days (subacute phase, n = 4) and ≥150 days after diagnosis (chronic phase, n = 6). The acute phase sample results (up to 10 days of diagnosis) disclosed levels of CP of 231.2 ± 23.3 ng/mL. Levels of 68.43 ± 16.6 ng/mL were detected during the subacute phase, and levels of 113.6 ± 55.6 ng/mL during the chronic phase. Our preliminary data indicate that high levels of CP can still be detected during the chronic phase of KD suggesting that an inflammatory process continues active. Further study on the presence sustained inflammatory process during KD evolution and on the use of CP as an inflammatory marker are needed and should be performed.
Robust and reliable Screening of HPV Subtypes by Mass Spectrometry

R. Birse1, P. Wandernoth2, M. Kriegsmann1, N. Arens2, R. Everts1, J. Kriegsmann1. 1Agena Bioscience, San Diego, CA, 2Molecular Pathology, Trier, Germany, 3Institute for Pathology, Ruprecht-Karls-University, Heidelberg, Germany

Background: High-risk Human Papilloma Virus (HPV) tests were recently approved by the U.S. Food and Drug Administration (FDA) as a primary screening tool for cervical cancer risk in women aged 25-65 years without a simultaneous Pap smear. It can be predicted that pathologists and molecular biologists will be faced with a significantly increased demand for molecular HPV testing in the near future. Consequently, tests systems will be required for the specific and sensitive detection of high- and low-risk HPV subtypes in a time and cost effective manner.

The mass-spectrometry-based MassARRAY System® (Agena Bioscience, Inc.) has the potential to meet all these requirements as it comprises a comprehensive set of tools to run multiplexed panels in clinical studies that achieve an unsurpassed level of specificity, sensitivity and sample throughput, at a low per sample cost.

Methods: We developed an assay for the MassARRAY to screen for the presence or absence of 19 specific HPV types plus a positive control housekeeping assay. The HPV panel consists of a single multiplex-reaction allowing minimal sample input. We compared the MassARRAY data to the results of hybridization assays (COBAS and Chipron). In total 45 formalin fixed paraffin embedded (FFPE) tissue samples and 10 Pap smear liquid samples were analyzed. Moreover we assessed three different DNA isolation methods.

Results: All high risk HPV subtypes detected by the COBAS or the Chipron system could also be detected by the MassARRAY HPV panel. Moreover, the MassARRAY panel outperformed the number of HPV subtypes detected when a high yield DNA extraction process was utilized.

Conclusion: We conclude that the MassARRAY HPV panel represents a highly specific, sensitive and reliable method for the detection of HPV subtypes in FFPE samples and Pap smears in a high throughput setting. The MassARRAY® System is For Research Use Only. Not for use in diagnostic procedures.

Clinical Utility of Interferon Lambda 3 and BAFF polymorphisms as predictors of treatment response in patients with HCV

N. H. Zakaria1, M. K. Ahmed2, E. A. Ahmed2, H. S. Maher3. 1Alexandria Faculty of Medicine, Alexandria, Egypt, 2Molecular Biology Department - Al Borg Medical Laboratories, Jeddah, Saudi Arabia, 3National Cancer Institute, Cairo, Egypt

The goal of treatment in patients with chronic hepatitis C virus infection is to eradicate hepatitis C virus (HCV) RNA, which is predicted by the attainment of a sustained virologic response (SVR). A SVR is associated with a 99 percent chance of being HCV RNA negative during long-term follow-up. Treatment with pegylated interferon 2a or 2b combined with Ribavirin (RBV) has been considered the standard of care (SOC) for HCV treatment. Approximately 50% of patients with HCV exhibit Mixed Cryoglobulinemia which is a benign but premalignant condition. Aim of this work was to evaluate the clinical utility of interferon Lambda-3 polymorphism rs12979860 and its association with ~871 C/T BAFF polymorphisms as predictors of viral clearance and treatment response as well as MC occurrence among HCV treated patients.

Patients: Analytical cohort study conducted on 100 HCV patients. They received pegylated interferon and ribavirin as treatment of choice. All the patients have signed an informed consent. Methods: HCV quantitation by COBAS AmpliPrep, TaqMan PCR, Genotyping of HCV using the Abbott Real-time HCV Genotype II. Levels of BAFF mRNA were determined by real-time quantitative PCR. Results: Males were more non-responder than females. The level of viremia showed that a total of 68 patients were responders; 23.5% with very low level of viremia (~200,000 U/ml) with the rest of responders had viremia level between 600,000 to 800,000 U/ml. All responders achieved RVR after 4 weeks and maintained e-RVR after 12 weeks and predict more than 90% SVR. IL-28B polymorphysim rs12979860 showed 68 patients who were responders; 22 (32.4%) of them had IL-28B genotype CC, 37 patients of them (54.4%) had CT type, and 9 patients (13.2%) had TT genotype. The other 32 patients who were non responders showed increasing in CT genotype in 81.2% and 8.8% for TT genotypes. There was statistically significant difference between IL-28B polymorphism C allele in responders group for treatment (MCP=0.002).

Serum fibrosis marker panels FIB-4 and APRI are statistically equivalent to AST alone at predicting liver fibrosis in a cohort of 1733 hepatitis C virus infected patients

J. C. Drexel1, S. Wi2, R. S. Dlott3, M. S. Petrie4, B. Fettermann1, T. S. Lorey1, S. I. Sco5. 1Kaiser Permanente Regional Labarotories, Richmond, CA, 2The Permanente Medical Group, Richmond, CA

Background: With the recent availability of direct-acting antiviral drugs for hepatitis C virus (HCV) capable of inducing sustained virologic responses, efficient tools are needed to rapidly identify higher stage HCV-infected patients to prioritize them for early treatment. Fibroscan (transient elastography) is less invasive than liver biopsy and compares well to biopsy, however, Fibroscan availability is limited. Compared to biopsy, several studies demonstrated favorable performance of non-invasive multi-analyte serum fibrosis marker panels FIB-4 (index derived from alanine aminotransferase [ALT], aspartate aminotransferase [AST], platelets, and patient age) and APRI (AST-to-platelet ratio index). Serologic tests capable of identifying cohorts with higher stages of fibrosis at the time of HCV diagnosis would greatly aid in prioritizing patients for treatment. Our objective was to validate FIB-4 and APRI for staging fibrosis in our HCV-infected population.

Methods: Fibroscan results from 1733 HCV-infected patients were mapped to an F0-F4 equivalent scale. AST, ALT, and platelet results obtained 5 months before or 1 month after the date of the Fibroscan were used to calculate FIB-4 and APRI scores.

Results: The areas under the receiver operating characteristic curve (AUC) for distinguishing severe (F3-F4) from mild-to-moderate fibrosis (F0-F2) were not significantly different between FIB-4 (0.76; 95% CI 0.73-0.78), APRI (0.77; 95% CI 0.74-0.79), and AST (0.74; 95% CI 0.72-0.77). The AUCs for ALT (0.69; 95% CI 0.66-0.71) and AST:ALT ratio (0.55; 95% CI 0.52-0.58) were significantly worse. (See Figure 1.)

Conclusion: We concluded that AST alone was as effective as FIB-4 and APRI at distinguishing severe from mild-to-moderate fibrosis. Using a cutoff of ≥56 IU/L (normal AST reference range is 10-40 IU/L), AST is 59% sensitive and 79% specific for identifying severe fibrosis and could be used to prioritize patients for early treatment. In contrast to published studies, we concluded that no test was sufficiently sensitive to rule out severe fibrosis.
# B-103

**Increase Prevalence of Extended-Spectrum Beta-Lactamase (ESBL) Producing Enterobacteria in Urinary Tract Infection in Long-Term Care facilities.**


**Background:** Urinary tract infection (UTI) is one of the most common infections in the geriatric population; it is considered the second cause of community-acquired infection and nosocomial infection. Most of the UTI in long-term care facilities are caused by gram negative bacteria; however, Extended-Spectrum Beta-Lactamase producing enterobacteria are a big concern in these facilities where they can spread rapidly where most of the residents are elderly, frail and are on multiple medications. In addition, identifying these organisms in urine culture is becoming a challenge to physicians by limiting their therapeutic options since they are resistant not only to extended-spectrum cephalosporins but other antibiotics such as aminoglycosides and sulfonamides.

**Design:** We analyzed data from 604,890 specimens collected for urine culture from 2006-2015 from residents in Long-Term Care facilities. Cultures were done utilizing MicroScan Walkaway 96 conventional panels. We calculated the prevalence of positive cultures and the prevalence of ESBL among the population tested. No growth and <10,000 colonies were considered negative, cultures with > 50,000 colonies were considered positive.

**Results:** More than 50 % of cultures were positive across the years tested, and >70% of the positive cases were due to gram negative bacteria. ESBL producing bacteria accounted for 15.2% of the gram negative bacteria and 11.4% of all positive culture in 2015; the number reflects more than double the results from 2006 (6.0% and 4.2% respectively).

**Conclusion:** Our results showed a progressive increase in ESBL producing enterobacteria in elderly patients and it supported the notion that Long-Term Care facilities could be a reservoir for these microbes. An infection control and antibiotic stewardship should be implemented to identify the risk factors for ESBL and optimize the use of antibiotics to improve patient outcome, limit the spread of these bacteria in the geriatric population, and reduce the cost of patient care.

---

# B-104

**cerebro spinal fluid adenosine deaminase levels in tuberculous and pyogenic meningitis**

S. D. Kolla1, J. R. Peela2, S. Panakala2, 1Department of Biochemistry, RangaRaya Medical College, NTR University of Health Sciences, Kakinada, India, 2Faculty of Medicine, Quest International University Perak, IPOH, Malaysia, 3Manamata Medical College, Khammam, India

**Background:** Tuberculosis is still leading cause of mortality and morbidity in India particularly in rural and tribal population. The annual death rate is more than 400,000 a year. Although there were several programmes like NTPC (National tuberculosis control programme) and RNTCP (Revised national tuberculosis control programme) still the the mortality is high. Mostly due to lack of early diagnosis and discontinuity of treatment. Tuberculous meningitis is one of the emergencies in these population especially in children which requires immediate hospitalization and treatment. Though there are diagnostic procedures like sputum AFB, CSF analysis and still needs better methods for diagnostic and prognostic use. The present study is determination of Adenosine deaminase enzyme which is important in purine degradation in tuberculosis meningitis patients and pyogenic meningitis patients before and after treatment.

**Methods:**

60 clinically symptomatic cases of meningitis admitted between 16 and 50 years of age in medical wards of King George Hospital, Visakhapatnam, India between January 1999 and November 1999 were enrolled the present study. Out of these 60 cases there was 23 cases with tuberculous meningitis, 9 cases were diagnosed as pyogenic meningitis and remaining with other causes which were not included in study. There were 12 normal adults were also in this whose diagnosis was not meningitis. Lumbar puncture was done on the day 1, day 8 and day 30 for tuberculous patients, day 1 and day 8 for pyogenic meningitis patients and was only once to the controls. CSF was collected and examined for levels of Adenosine Deaminase by authentic method. Anti tuberculous treatment was started for tuberculous meningitis patients and anti bacterial treatment was started for pyogenic meningitis patients.

**Results:** The levels of adenosine deaminase significantly high in both pyogenic and tuberculosis patients when compared with controls p-value < 0.05 (3.86+/-.15). The levels of adenosine deaminase was significantly decreased on day 8 after treatment p-Value < 0.05(13.04+/-.1.74 on day 1 and 9.4+/- 1.2 in day 8). In tuberculous meningitis it was the levels of adenosine deaminase levels were significantly elevated on day 8 after treatment (p-value < 0.05) and significantly reduced on day 30 of the treatment (p-value < 0.05) ( 15.08+/-.3.15 on day 1 ,17.99+/-.4.81 on day 8 and 9.4+/- 1.9 on day 30).

**Conclusion:**

The present study the therapeutic response of pyogenic meningitis was immediate according to the levels of adenosine deaminase and is delayed in case of tuberculosis. Hence this ADA activity is useful tool in bacterial meningitis as well as in tuberculous meningitis.

---

# B-105

**Use of AccuPlex Recombinant Sindbis Virus Technology to Produce a Noninfectious, Whole Process Zika Control**

R. Vemula, C. E. Huang, B. Anokell, SeraCare Life Sciences, Gaithersburg, MD

**Background:** An outbreak of the mosquito-borne Zika virus occurred in Brazil in spring of 2015. Since that time, the virus has gained global attention due to its rapid spread through at least 21 countries of the Americas and its possible link to neurological birth defects. In response to this outbreak, diagnostic laboratories and test developers need to design, manufacture and validate molecular diagnostic assays and this requires stable, reproducibly manufactured positive reference materials. SeraCare has developed the AccuPlex™ Zika Positive Reference Material using recombinant Sindbis virus technology. This well established recombinant viral technology has many advantages as a NAT quality control material: it mimics clinical samples because it undergoes the entire extraction procedure; it is non-infectious and ensures biological safety for lab personnel; and it has extended stability at 2-8 ºC and does not require freezer storage.

**Methods:** The Zika virus is a positive sense RNA virus whose genome is approximately 10.7 Kb. The Zika genome was divided into four (4) segments and each segment was used to generate an AccuPlex™ recombinant virus using Sindbis vector system. Construct #1 contains Capsid (C), precursor Membrane (M), envelope (E), and non-structural protein 1 (NS1) genes. Construct #2 contains NS2A, NS2B, and NS3 genes. Construct #3 contains NS4A and NS4B, and Construct #4 contains NS5 gene. Each construct is designed to contain ~150 bp of sequence which overlaps with the other constructs to assure that a mixture of the recombinant viruses is compatible with all Real Time PCR targets (even those that may span the junction between genes). Each construct was used for in vitro RNA transcription, and RNA was introduced into BHK-21 cells where recombinant Sindbis virus particles were assembled. The viruses were heat inactivated and purified. The strategy of dividing the Zika genome into four different recombinant viruses such that each recombinant virus is not functional assures the safety of the reference material and the heat inactivation serves as an additional safety precaution.

**Results:** Initial titering of the purified viral supernatant used digital PCR analysis and either primers/probes specific for the Sindbis vector or primers/probes to the Zika envelop region as in Lanciotti et al. 1 The recombinant viruses were mixed and diluted...
Infectious Disease

into defibrinated human plasma at a concentration of 1.0E+05 copies/mL. Ongoing accelerated and real time stability studies indicate that AccuPlex™ recombinant viruses are stable at room temperature for at least 17 months and at 4 °C for at least two years. Accuracy and precision data on PCR based assays will be presented.

Conclusions: SeraCare has developed a stable, well-characterized whole process control for Zika virus. This reference material will enable laboratories to validate tests and train technicians to ensure preparedness. The Accuplex™ Zika product demonstrates the utility of recombinant virus technology to produce non-infectious controls for biochemical assays that are difficult to source or propagate.


EVALUATION ANTIFUNGAL ACTIVITY OF DRUGS ANTIFUNGAL AND CRUDE EXTRACTS FROM BRAZILIAN CERRADO PLANTS AGAINST CLINICAL ISOLATES CANDIDA SPECIES


Most cases of systemic candidiasis were assigned to the Candida albicans species. However, non-albicans Candida have been identified as human pathogens in infections representing a change in prevalence with a percentage at times related to Candida albicans. This change in the etiology of systemic candidiasis is related to new diagnostic methods, such as identification of species using molecular techniques and may reflect on the high rate of resistance to antifungal Candida non-albicans. This study’s main proposed objective is to characterize clinical isolated cases of Candida spp. to epidemiological and to search species resists to antifungal agents during treatment. Plant extracts of Brazilian Cerrado were used to evaluate. Microbiological identification on non-molecular and molecular methods, and disk diffusion test were used as a protocol reference. The microbiological identification methods characterized 150 isolated clinical in patients suspected of systemic infection in the Federal District, Brazil, between January/2011 and December/2012. Candida albicans is the most commonly isolated species (50.6%), followed by Candida parapsilosis (21.3%), Candida tropicalis (16.6%), Candida glabrata (8.0%), Candida krusei (0.7%), Candida guilliermondii (0.7%), Candida intermedia (0.7%) and Kadaamae ohmeri (0.7%), which were isolated in bronchoalveolar lavage samples, blood and mucus, predominantly in male patients aged between 21 and 60 years. The disk diffusion test was used to determine the antifungal activity of conventional agents and plant extracts. In conventional antifungal agents, most isolated clinical cases of Candida were sensitive to amphotericin B (97.9%); 91.9% were susceptible to voriconazole; 91.3% to fluconazole; 73% itraconazole and 2.7% 5-fluorocytosine. The resistance to azoles has been demonstrated by isolated cases of C. glabrata and 5-fluorocytosin for all Candida species. When evaluating the antifungal activity of the extracts, the six species of plants, Eugenia dyssenterica, Pouteria ramiflora, Pouteria torta, Erythroxylum subrotundum, Erythroxylum daphnites and Bauhinia rufa showed inhibitory effect on various Candida species, and Candida glabrata the most inhibited species. Our results suggest new therapeutic targets with potential antifungal activity in extracts evaluated primarily on Candida glabrata.

B-106

Comparing Plasma and Urine Parallel Samples for the Diagnosis of Zika Virus by Qualitative Real-Time PCR


Background: Since May 2015, Brazil Ministry of Health confirmed a ZIKV outbreak in the region where A. aegypti is widely distributed. Furthermore, sexual and blood transfusion-transmission have recently been reported, in addition to transplacental transmission. Several cases of the current newborn microcephaly outbreak in Brazil are potentially linked to ZIKV epidemics besides increased frequency of Guillain-Barré syndrome, also associated to ZIKV infection. However there are no specific symptoms for Zika fever, which may be confused to other arboviroses affecting Brazil at the present moment. These concurrent outbreaks call for accurate diagnostic tools of limited availability. The best way to diagnose Zika infection is during the acute phase detecting viral RNA in plasma (0 to 5 days after symptoms onset) and urine (6 to 15 days after onset) by reverse transcriptase PCR (RT-PCR). Serological diagnosis might be misleading due to cross-reactivity of ZIKV with other flaviviruses such as Dengue 1 - 4. Objective: The aim of this study was to compare ZIKV detection in plasma and urine samples from patients from a large Brazilian private laboratory using qualitative Real Time PCR. Methods: 371 Plasma samples and 177 urine samples were collected from patients, RNA automated extraction was performed using QIAasymp Phyto/bacteria mini kit (Qiagen), RT-PCR was performed with High Capacity DNA Reverse Transcription Kit (Thermo Fisher) and the viral regions of interest were amplified with two different assays (Lanciotti RS et al, 2008) by TaqMan Real time PCR (Thermo Fisher). RNaseP mRNAs was used as internal control. Samples were classified as “detectable” when both primers were amplified, “undetectable” when there was no amplification for both primers and “inconclusive” when amplification was observed for only one assay or if the result was below the limit of detection of the test (plasma: 820 copies/mL; urine: 160 copies/ mL). Real time PCR for inconclusive plasma samples was also performed in urine to assure the result. Results: From the 548 samples, 116 samples of plasma and urine were collected simultaneously, in the other 432, urine was solicited on demand to assure the result. A total of 44 samples were considered reactive (8.02%). Among the detectable samples, ten were undetectable or inconclusive in plasma but detectable in urine (22.7%), five were detectable in plasma and undetectable or inconclusive in urine (11.4%) and six were undetectable in plasma and inconclusive in urine (13.7%) while 5 showed concordance in between the two biological fluids (11.4%). Conclusion: According to our results, the use of urine samples increased the rate of molecular detection of ZIKV and should be considered when blood collection is difficult to be assessed. Furthermore, despite urine diagnosis may not be of clinical

B-107

Molecular Epidemiology of the Clonal Relationship among Klebsiella pneumoniae Carbenapenemase Strains recovered from Six Brazilian Hospitals using DiversiLab System

J. Monteiro, F. M. Inoue, J. L. Costa, A. P. T. Lobo, M. C. Feres, S. Tufik. ‘Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil, ‘bioMerieux, Sao Paulo, Brazil

Background: The emergence and intra-hospital dissemination of multidrug-resistant Gram-negative microorganisms and carbapenemases producers have been described in Brazil, as well as in many parts of the world. The inter-hospital evaluation of the relationship among Klebsiella pneumoniae carbapenemase (KPC) recovered from different hospitals could be helpful to understand the dynamic of the resistance dissemination in a specific region. The aim of this study was to evaluate the relationship among KPC samples isolated from several Brazilian public hospitals represented by the North, South and East regions of the São Paulo city. Methods: Between November and December 2014 was selected a group of K. pneumoniae strains resistant to cephalosporins and carbapenems. The samples were isolated from six different public hospitals of the São Paulo city. The hospitals were identified by the letters HA to HF. Eight samples per hospital and only one per patient was included in this study. The bacteria identification was performed using mass spectrometry (Vitek-MS, bioMerieux) and the minimal inhibitory concentration (MIC) of antibiotics were determined using the Vitek 2 System (bioMerieux). The presence of carbapenemases (KPC, VIM, IMP, NDM and OXA-48) and blaCTX-M gene were determined by PCR using specific primers for each gene. The genetic relatedness of the strains was characterized by automated repetitive extragenic palindromic polymers chain reaction (Rep-PCR) based DiversiLab system (bioMerieux) and the analysis of the profiles was performed using Pearson’s Correlation in the DiversiLab software (version 3.4). Isolates with similarities of < 95% were considered different, and isolates with similarities of >97% were considered indistinguishable. Isolates with similarities of >95% and >97% were categorized manually using the pattern overlay of the analysis tool in the software. Results: All 44 samples were identified as Klebsiella pneumoniae and all of them, were detected high level of resistance to imipenem and meropenem (MIC; ≥ 4 μg/mL) according to the CLSI 2014 guidelines. The presence of the blaKPC and blaCTX-M genes was detected in 44 and 33 samples, respectively. The rep-PCR analysis identified five different clusters (C1 to C5) and 11 patterns among the 44 isolates. Three of the five clusters (C3, C4 and C5) were identified in only one sample, each and represent three different hospitals (HA, HB and HF). On the other hand, cluster C1 showed two patterns and were identified in 15 isolates recovered from four hospitals (HB, HC, HD and HE). The cluster C2 showed six patterns and were identified in 26 isolates recovered from all six hospitals (HA, HB, HC, HD, HE and HF). The clusters C1, C2, C3, C4 and C5 showed a pattern of similarity <75%. Conclusion: Our results reveal the prevalence and the spread of two main clusters (C1 and C2) of KPC in different hospitals and regions of São Paulo city. These findings reinforce the continued need for infection control, antibiotic management and application of an intra- and inter-hospital surveillance system.

68th AACC Annual Scientific Meeting Abstracts, 2016 S175
Detection of NDM-1 and CTX-M-type producing Enterobacteriaceae strains in Brazil

J. Monteiro, F. M. I. Neto, A. P. T. Lobo, M. C. Feres, S. Tufik. Associação Fundo de Incentivo a Pesquisa, São Paulo, Brazil

Background: New Delhi Metallo-β-Lactamase (NDM) has become one of the main resistance mechanisms in Brazil, especially in Enterobacteriaceae, worldwide, with reports of cases. The objective of this study was to report the detection of NDM-1 producing Enterobacteriaceae clinical isolates recovered at Brazilian hospitals.

Methods: In January 2015 and in January 2016 five carbapenem-resistant Enterobacteriaceae strains were isolated from five patients hospitalized in two tertiary Brazilian hospitals. These strains were isolated from Urine (n=2), catheter tip (n=1), and rectal swab (n=2). The bacterial identification was performed using mass spectrometry (Vitek-MS, bioMérieux) and the minimal inhibitory concentration (MIC) of antibiotics were determined using the Vitek 2 System (bioMérieux), as well as E-test method, according to CLSI guidelines. The screening test of carbapenemase was performed by phenotypic assay using commercially available disks containing carbapenems with and without EDTA (0.1 M), clavulacin (75 mg/mL) or phenylboronic acid (40 mg/mL), as recommended by the Brazilian National Health Surveillance Agency (ANVISA). Then, the detection of the carbapenemase (blaNDM, blaCMR, blaVIM and blaTEM) and ESβL genes were analyzed by Sanger sequencing analysis of the genes. Genetic relatedness of the strains was characterized by Pulsed-Field Gel Electrophoresis (PFGE) using the Specl restriction endonuclease. Results: Among the five Enterobacteriaceae studied, three isolates were identified as Providencia rettgeri and two as Klebsiella pneumoniae. In all of them, high-level of resistance were detected to ceftazidime (MIC, >16 µg/mL), ceftriaxone (MIC, >4 µg/mL), cefepime (MIC, >16 µg/mL), etepenem (MIC, >2 µg/mL), imipenem and meropenem (MIC, >4 µg/mL). One of K. pneumoniae strains was also resistant to amikacin (MIC, 32 µg/mL) and colistin (MIC, >16 µg/mL). All strains showed the diameter-zone difference >5 mm between the carbapenem/ EDTA and carbapenem disks, screened as possible metallo-β-lactamase (MβL) producers. The presence of blaNDM and blaVIM genes was detected in all strains. All P. rettgeri strains showed an identical PFGE pattern. The PFGE protocol to K. pneumoniae strains is running. Conclusion: An early detection by screening methods could be very helpful for surveillance and infection control measures. In addition to that, the findings of the identical PFGE pattern among P. rettgeri suggest the occurrence of a horizontal dissemination and an emergence of this important mechanism of resistance in Brazil.

Comparison of DiversiLab System for Microbial DNA Typing with Pulsed Field Gel Electrophoresis (PFGE)


Background: Hospital-acquired infections due to bacteria that are resistant to multiply antibiotics have led to a worrying situation in many parts of the world. The control of nosocomial infections is based, in part, on tracking the spread of isolates potentially responsible for outbreaks. Due to its high discriminatory power, pulsed-field gel electrophoresis (PFGE) is considered as the reference method for molecular typing of many bacterial. However, this technique has major limitations: it is time-consuming, labor-intensive, technically variable, which affects reproducibility and provides subjective-interpretable results. DiversiLab® System (Biomerieux) is rapid and semi-automation technique for typing microbial strains based on repetitive-PCR technology (Rep-PCR), which primers are complementary to non-coding repetitive sequences found in microbial genome. Biomerieux portfolio presents different species specific-kits.

Objective: The aim of this study was to assess the DiversiLab (DL) system for typing same bacterial pathogens causing hospital-associated outbreaks by evaluation and comparing its performances with PFGE results. Methods: 4 strains of Acinetobacter baumannii and 4 Klebsiella pneumoniae were evaluated by DL (generic) kit and species specific kits. 2 strains of Stenotrophomonas maltophilia and 4 Pseudomonas aeruginosa were evaluated only with DL Bacterial generic kit. DNA from bacteria cultures was extracted with MoBio Ultra Clean Extraction Kit (MoBio) and rep-PCR was performed with the respective DL system kit including positive controls. Chips (Biomerieux) containing rep-PCR products were inserted into the Bioanalyzer (Agilent) and the results analyzed by DL software. Results: Results by DL Bacterial Kit agreed to those generated by PFGE for typing of S. maltophilia and P. aeruginosa strains, suggesting that S. maltophilia samples were indistinguishable from each other and P. aeruginosa were different between them. When PFGE was compared to DL Bacterial (generic) kit and DL Acinetobacter kit, our data showed that PFGE results were similar to DL Acinetobacter kit results, suggesting that these strains were indistinguishable. PFGE results also corroborated with those generated by DL Klebsiella kit, showing that 2 strains were similar to each other, and the other 2 K. pneumoniae were different. In both cases, DL Bacterial (generic) kit generated different results from PFGE. Our data show that DL results were in accordance to PFGE results, validating DL system with as reliable to perform bacteria typing.

Conclusion: In summary, DL is a useful and fast tool to help identify hospital outbreaks of A. baumannii, S. maltophilia, K. pneumoniae and P. aeruginosa.
Clinical Performance of Elecsys® Anti-HCV II in Subjects with Increased Risk of Hepatitis

T. Eden, R. Ostlund Jr., P. Jones, S. Jortani, M. Lessig, E. Schiff, J. Layton, R. Dworschack. Core Laboratory for Clinical Studies, Washington University, St. Louis, MO, 2Southbend Medical Foundation, Southbend, IN, 3Kentucky Clinical Trials Laboratory, Louisville, KY, 4University of Louisville, Louisville, KY, 5Formerly Nationwide Laboratory Services, Fort Lauderdale, FL, 6University of Miami, Miami, FL, 7Roche Diagnostics Inc., Indianapolis, IN

A multicenter clinical performance study of liquid Elecsys® Anti-HCV II immunoassay on cobas e 601 analyzer was recently completed. Study population consisted of adult and pediatric subjects at risk for hepatitis (sexual practice, behavior, medical status or occupation). Elecsys® assay is an automated sandwich immunoassay based on the chemiluminescence principle where complexes of sample anti-HCV antibodies, biotinylated1–ruthenylated-antigens (core, non-structural NS3/NS4), and streptavidin-magnetic microparticles are captured on an electrode. The primary objective was to evaluate percent agreement between Anti-HCV II and reference assay. The secondary objectives included evaluation of hepatitis A/B co-infections, non-HCV Flaviviridae viruses, anti-HCV antibody genotype recognition, seroconversion sensitivity, and determining imprecision.

Specimens (serum, sodium citrate plasma) were tested in four US sites using Elecsys® Anti-HCV reference assay. Final HCV interpretation was based on a testing algorithm for reference reactive samples: two comparator immunoassays, Abbott Architect Anti-HCV and ORTHO VITROS® (HCV), and HCV RNA determination (Roche AMPLICOR Hepatitis C assay) for comparator-indeterminate samples. Positive /negative percent agreements with 95% confidence limits are listed below.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult</td>
<td>560</td>
<td>1683</td>
</tr>
<tr>
<td>Pediatric</td>
<td>98.81%</td>
<td>97.17-99.27%</td>
</tr>
<tr>
<td>Pregnant</td>
<td>97.95±2%</td>
<td>97.34-99.99%</td>
</tr>
</tbody>
</table>

Twenty-nine Flaviviridae (West Nile Disease, Dengue Fever, Murray Valley Encephalitis, Kunjin Fever) and 289 other specificity specimens representing 28 diseases were concordant between both assays. Seroconversion sensitivities in both assays were equivalent in ten commercial panels; Elecsys® Anti-HCV II converted earlier in six panels and later in one panel. Both assays demonstrated equivalent genotype recognition in three genotype panels. Imprecision (CLSI EP5-A2) was evaluated using three reagent lots and two operators in three US sites. Three replicates imprecision pools were tested in two runs/day for five days. All %CVs for Repeatability and Reproducibility were <3.1% and <12.0%, respectively. The C5–C95 (CLSI EP12-A2) interval ranged from 0.97–1.06 COI.

Evaluation of the Elecsys® Anti-HCV II assay / cobas e 601 analyzer demonstrated acceptable clinical/analytical comparison between lyophilized first generation and second generation liquid reagent.

Recombinant chimeric IgG/IgM mouse-human antibodies for the manufacture of calibrators and controls for diagnostic test kits.

D. Shan, R. MacDonald. CTK Biotech, San Diego, CA

Recombinant chimeric antibodies containing murine variable regions and human constant regions are good candidates as calibrators or controls in immunoassays when positive human samples are difficult to obtain. Dengue was selected as a model system. We chose to use a diagnostic kit, the OnSite Dengue IgG/IgM Combo Rapid Test (R0061C), which is designed to detect and discriminate the IgG/IgM subtypes against the Dengue virus envelope protein in human serum or plasma. A monoclonal antibody against a specific site in the dengue envelope protein which is conserved in all four types of the dengue virus was developed from murine. This antibody was used to generate humanized, chimeric antibodies carrying the target binding site. First, heavy-and light-chain variable-region genes were cloned from the hybridoma cells and transferred into immunoglobulin expression vectors containing human kappa and IgG1 or IgM constant regions. The constructs were then used to transiently transfected COS cells, and the chimeric antibodies that were expressed were tested by ELISA for target-binding activity. After confirmation of the binding activity, CHO cells were stably transfected with these constructs. Chimeric IgG/IgM antibodies purified from the CHO cell culture media were tested using the OnSite Dengue IgG/IgM Combo Rapid Test kit. A strong test line density was observed for both chimeric IgG and IgM antibodies, which was comparable to the results using positive patient samples. The immunoreactivities of both chimeric IgG and IgM antibodies were also confirmed by ReombiELISA Dengue IgG and IgM ELISA tests. These data demonstrate that chimeric mouse-human antibodies are a feasible alternative to high-titer positive human samples for the manufacture of calibrators and controls for diagnostic test kits.

Evaluation of Serum Vaspin & Lipid Hydroperoxide Levels in Patients with Leishmaniasis

H. Sezen1, E. Savik1, H. Bilinc1, M. Aksoy2, N. Aksoy1, 1Biochemistry, Sanliurfa, Turkey, 2Dermatology, Sanliurfa, Turkey

Background A new adipokine Vaspin is released from adipose tissue. The function of it is poorly understood in the body. It was shown that it takes place in an inflammatory process and insulin resistance. It has been claimed that it plays a compensatory role in inflammation caused by obesity. It also inhibits the expression of TNF-induced intracellular adhesion molecule (ICAM)-1 by lowering reactive oxygen species (ROS). Lipid hydroperoxide (LOOH) is the first product of lipid peroxidation and shows intense oxidative stress. Both Vaspin and LOOH levels previously have not been evaluated in patients with Leishmania which causes an increase in oxidative stress and inflammation. The aim of this study was to evaluate the Vaspin and LOOH levels of Leishmania patients. Methods The study was conducted on 35 active Leishmania patients and 35 healthy volunteers. Venous blood samples were taken from patients and controls after overnight fasting and were centrifuged and then serum was separated. Vaspin was studied by ELISA method and LOOH by spectrophotometric method. Results Sex was similar between patient and control group (p>0.05). Age was higher in the patient group than in the control group (p<0.019). Serum vaspin levels were lower in the patient group than in the controls (p<0.001 and Figure 1a) whereas LOOH was higher (p<0.001 and Figure 1b). LOOH was negatively correlated with Vaspin (B corr. Coef. -0.299 and p<0.015). Multivariate logistic regression analysis showed that the only factor independently affecting existence of the disease is LOOH. Conclusion This study data showed that LOOH which is an indicator of oxidative stress and lipid peroxidation increased in Leishmania patients. In contrast, the levels of Vaspin which is thought to trying to suppress inflammation decreased.