Predicting liver fibrosis staging using noninvasive biomarker M2BPGi in patients with hepatitis B infection

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**Background:** Liver fibrosis is the common consequence of chronic hepatitis B virus infection, leading to the formation of cirrhosis. Accurate assessment of liver fibrosis is specifically essential for the management of patients with liver cirrhosis accompanied by distortion of hepatic vasculature. Currently liver biopsy is widely used, but it has many limitations, for instance, subjectivity in reporting, high costs, risks of bleeding and pneumothorax, and discomfort to patients. Thus, a novel serum glycomarker, M2BPGi, has been newly developed. The present study aimed to investigate the diagnostic performance of M2BPGi in HBV infected patients by comparing with other noninvasive methods such as AST-to-platelet ratio (APRI), FIB-4, AST to ALT ratio (AAR), and RDW to platelet ratio (RPR), in order to assess the utility of M2BPGi as a liver fibrosis stages monitoring tool.

**Methods and results:** Serum samples were collected from 228 HBV infected patients. Liver fibrosis stages of all patients were diagnosed using FibroScan, with 127 patients (55.7%) had fibrosis of F0-1, 32 patients (14.04%) of F2-3, and 69 patients (30.29%) of F4. Median M2BPGi values in each fibrosis stage were: 0.88 cut-off index (COI) in F0-1, 1.165 COI in F2-3, and 1.92 COI in F4, respectively. Furthermore, as for diagnosing significant fibrosis (≥F2), the areas under the receiver operating characteristic curve (AUC) of M2BPGi (0.788) was comparable to FIB-4 (AUC=0.820), APRI (AUC=0.817) and RPR (AUC=0.799), but significantly superior to AAR (AUC=0.585) (Figure a). In addition, M2BPGi yielded the highest AUC of cirrhosis (≥F4) (AUC=0.811) compared with APRI (AUC=0.809), FIB-4 (AUC=0.799), AAR (AUC=0.560) and RPR (AUC=0.786) (Figure b).

**Conclusion:** An increasing trend in M2BPGi levels associated with the progression of liver fibrosis in HBV infected patients was observed. M2BPGi can be served as a potential noninvasive glycomarker to assess the stage of liver fibrosis, especially for patients with F4 HBV fibrosis.
Infectious Disease

was 0.9917, 0.9912, 0.9869, 0.9910 and 0.9906 for serum HbsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-HBc detection.

Conclusions: HYBOMI quantitative detection reagents for HBV serological markers, with HYBOMI CLIA platform AE-240, present an excellent quantitative analysis performance and a good correlation with reference reagents in clinic, which offers a rapid and accurate technology for HBV serological tests and may be useful for clinical surveillance and HBV infection research.

B-061

The establishment of non-invasive diagnosis of liver fibrosis model in chronic hepatitis C in Chinese people

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OBJECTIVES: Noninvasive markers of liver fibrosis in patients with chronic hepatitis C are needed for predicting disease progression. We aimed to establish a non-invasive diagnostic model to predict liver fibrosis development in hepatitis C virus (HCV)-infected patients in China. METHODS: One hundred and seventy-five patients with chronic HCV infection without other potential risk factors who underwent liver biopsy and serological tests were enrolled; factors evaluated included age, sex, viral load, fibrosis stage, aspartate and alanine aminotransferase, alkaline phosphatase, γ-glutamyltransferase, total protein, albumin, fasting glucose, total, bilirubin, direct bilirubin, total bile acids, creatinine, total cholesterol, triglycerides, platelet count, prothrombin time, thrombin time, activated partial thromboplastin time, hyaluronidase, type III collagen N-telopeptide, laminin, type IV collagen, anti-HCV IgG. For the formulation of diagnostic model, univariate logistic regression analysis was performed on variables between patients in the training set. Significant variables from the univariable analysis (P <0.05) were then subjected to multivariate logistic regression analysis to identify independent factors associated with fibrosis. The fibrosis index derived from the training set was then applied to the validation set to test the predictive power of the selected model. Clinical data were compared with those for other noninvasive models (AST-to-platelet ratio (APR), AST to ALT ratio (AAR), age-to-platelet ratio (API), cirtirhus discriminate score (CDS), FIB-4, and Forn’s index) for estimating liver fibrosis using receiver operating characteristic (ROC) analysis. ROC curves were constructed to measure the diagnostic capacity of each test. RESULTS: The multivariate logistic regression analysis identified alanine aminotransferase (ALT), total bile acids (TBA) and hyaluronidase (HA) as independent risk factors for fibrosis. The model of ATH is consist of 3 indicators, ALT, TBA and HA, ATH= ln (ALT) × ln (TBA)× ln (HA). ATH was independently associated with liver fibrosis stage as determined by liver biopsy(r = 0.716, P < 0.01). The cut-off values of ATH for fibrosis stages ≥S2, ≥S3, and S4 were 3.71, 4.72, and 6.21, respectively. The area under the receiver operating characteristic curve values (AUROC) of ATH for significant fibrosis (≥S2), severe fibrosis (≥S3), and cirrhosis (S4), were 0.877, 0.897, and 0.841, respectively. The AUROC of ATH model was significantly higher than alone ALT, TBA, HA index in fibrosis stages of significant fibrosis, severe fibrosis, and cirrhosis, respectively (P < 0.01). ATH values offered a superior AUROC curve for the diagnosis of significant fibrosis, severe fibrosis and cirrhosis compared with the APRI, CDS, API, AAR, FIB-4 and Forn’s index. Compared with the other noninvasive models s and scoring systems, ATH was the most useful marker for differentiating between fibrosis stages. For predicting liver cirrhosis (S4), ATH model had the highest AUROC (AUCATH=0.841, P<0.001) and for predicting severe liver fibrosis (≥S3), ATH model had the highest AUROC (AUCATH=0.897, P=0.001) and for predicting significant liver fibrosis (≥S2), ATH model also had the highest AUROC (AUCATH=0.877, P<0.001). CONCLUSION: The ATH model may be a simple, reliable, and non-invasive method to evaluate liver fibrosis in HCV-infected patients in China without an unnecessary liver biopsy.

B-062

BK virus-IgG and BK virus-specific ELISPOT assay in healthy donors and pretransplant recipients

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Background: BK virus (BKV) is widespread in the human population, but rarely induced apparent disease in healthy individuals. However, the reactivation of BKV is major infectious complication in kidney transplant patients that can lead to graft loss. The aim of this study was to investigate the BK virus-specific cellular and humoral immunity in healthy controls and pretransplant chronic kidney disease (CKD) patients.

Methods: Peripheral blood mononuclear cells and sera were collected from 44 healthy individuals (M=23.21, age 35.8±11.2) and 26 CKD patients (M=13.13, age=45.7±11.6). BKV specific IgG levels were measured using qualitative Human BK virus IgG ELISA kit (MyBioSource, USA). The IFN-γ ELISPOT assays were performed to measure BKV-specific T cells (spots per 3x10⁶ lymphocytes) following the stimulation with different BK virus antigens (Large T antigen, Small T antigen). The ELISPOT results were more frequent in CKD patients versus healthy controls (LT, 76.9% vs. 47.7%, P=0.017; ST, 73.1% vs. 29.3%, P=0.001; VP1, 69.2% vs. 25.0%, P=0.001; VP3, 73.1% vs. 31.8%, P=0.001). LT and VP1 antigens were the most immunogenic proteins showing significantly higher ELISPOT results in healthy individuals (P<0.001, 0.001). In addition, LT and VP1 antigens induced a wide range of ELISPOT results in CKD patients (0.833 and 0.717 spots/3x10⁶, respectively). However, BKV-ELISPOT results were not different between individuals with BKV-IgG+ and BKV-IgG- (P=0.05). In BKV-ELISPOT (+) results, the responses to different BKV antigens were highly heterogeneous, and the positive ELISPOT reactions to all five BK antigens were more frequent in CKD patients compared to healthy controls (53.9% vs. 11.4%, P<0.001).

Conclusion: BKV-specific IFN-γ ELISPOT responses were not associated with BKV-IgG and presented the increased activity and wide spectrum of responses in CKD patients. It might be a useful tool to monitor the viral replication and to guide immunosuppression.

B-064

Clinical benefits of the FilmArray GI Panel in an academic medical center

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Introduction Diarrheal diseases are a major cause of emergency department (ED) visits and hospitalization. Conventional methods for identification of gastrointestinal pathogens are time consuming, expensive, and have limited sensitivity. Patients may not receive antibiotics in a timely manner, may undergo unnecessary diagnostic testing, and incur excessive healthcare costs. The aim of this study is to determine the clinical impact of a new comprehensive molecular panel, the FilmArray® Gastrointestinal (GI) Panel (BioFire Diagnostics, Salt Lake City, UT) which tests for nearly all known agents of infectious diarrhea in a single hour. It is hypothesized that use of the GI Panel resulted in shorter length of ED or hospital stay and more appropriate time in isolation. Also, we sought to determine if the GI Panel lessened the need for other, more invasive and expensive tests, such as abdominal imaging studies.

Methods Following approval from the University of Florida Health Institutional Review Board (IRB), patients with stool cultures submitted between 6/1/16 and 12/31/16 that were processed via the BioFire GI Panel (n=123) were included as cases within this study. Following approval from the University of Florida Health Institutional Review Board (IRB), patients with stool cultures submitted between 6/1/16 and 12/31/16 that were processed via the BioFire GI Panel (n=123) were included as cases within this study. A historical control group (n=594) was obtained from the same time period a year prior to control for seasonality in GI illness, 6/1/15 to 12/31/15. Both the cases and controls were filtered to include only patients with a length of stay (LOS) that was 14 days or fewer.

Results A total of 123 patients were tested on the GI Panel. The organisms detected were norovirus (n=10), Salmonella (n=7), Shigella/EIEC (n=2), Campylobacter (n=2), sapovirus (n=3), rotavirus (n=1), Giardia lamblia (n=2), Vibrio cholera (n=1), Cyclespora cayetanensis (n=1), Adenovirus (n=1), Enterocaggregative E. coli (n=1), Enteropathogenic E. coli (n=6), and Enterotoxogenic E. coli (n=1). These patients were compared with 594 historical controls who were tested using conventional stool test methods. 30/123 (24.4%) of cases were positive for at least one organism other than C. difficile compared with 41/594 (6.9%) of control patients. Two or more non-C. difficile organisms (co-infections) were identified in 4.9% (n=6) of patients who were tested on the GI Panel but no co-infections were found in our control population. Patients tested on the GI Panel had an average of 2.6 other infectious stool tests whereas the control population averaged 3.02 additional stool tests (p < 0.05). The number of abdominal radiologic exams was also slightly lower in the GI Panel group.
Infectious Disease

B-066

Reevaluation of enzyme linked fluorescent immunoassay comparing with PCR assay for detection of Clostridium difficile toxins

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Background: Enzyme immunoassay capable of detecting both toxin A and B of Clostridium difficile (C. difficile) is routinely tested in hospital for the diagnosis of C. difficile associated disease. Recently, PCR test for the gene of C. difficile toxin B in diarrhea stool specimen is becoming popular in the diagnosis of C. difficile associated disease. Therefore, we compared the results of two methods in detection of C. difficile toxins.

Methods: In total, 643 diarrhea samples that were simultaneously analyzed for toxin A and B by enzyme linked fluorescent immunoassay (VIDAS CDAB, Bio-Merieux sa, France) and for C. difficile toxin B by PCR using the Seeplex Diarrhea ACE Detection kit (Seegene, Korea) in Cheju Halla Hospital from March 2015 to December 2016. Retrospectively we investigated the positive detection rates, concordance rate according to the level of relative fluorescence value in ELFA

Results: The positive rate was 8.55% when we regard “equivocal” as “positive” in ELFA, 5.8% when we regard “equivocal” as “negative” in ELFA, 8.8% in PCR. The concordance rate between ELFA and PCR was 94.4%. Nineteen (3.3%, 19/588) samples that were negative in ELFA were positive in PCR. Nine (50%, 9/18) samples that were equivocal in ELFA were positive in PCR, Eight (21.2%, 8/37) samples that were positive in ELFA were negative in PCR.

In the test of enzyme linked fluorescent immunoassay, the cut-off level in Relative fluorescence value (RFV) between “Negative” and “equivocal” and between “equivocal” and “positive” is 0.13, 0.34. ROC curve showed that the cut-off of 0.04 was 0.892 in Area under the curve (AUC) with 82.5 in sensitivity, 88.9 in specificity.

Positive rates of C. difficile by PCR is 1.9% under 0.04, 27.9% over 0.04 in RFV

Conclusions: The positive detection rates of PCR were higher than those of ELFA for the detection of C. difficile associated disease, hence the PCR assay for detection of toxin B is recommended especially in case ELFA is negative. PCR test is more useful to detect the causative agents including C. difficile toxin B. Re-set of Cut off limit in RFV in ELFA should be re-set based on the result of PCR test for C. difficile toxins.

Key Words: Clostridium difficile toxin, PCR, enzyme linked fluorescent immunoassay

B-067

Evaluating a Novel Host-immune Based Assay For Distinguishing Bacterial From Various Viral Infections In Febrile Children

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Background: A major challenge in effective management of febrile children is the clinical difficulty of distinguishing bacterial from viral infections. This uncertainty drives antibiotic misuse, hampering patient care and contributing to emergence of antibiotic resistance. ImmunoXpert™ is a novel assay that distinguishes bacterial from viral infections based on the serum levels of three host-proteins (TRAIL, IP-10, and CRP). Here we evaluated the assay’s ability to assign correct infection classification (viral or bacterial) in children infected with ten different types of viral strains as well as in bacterially infected children.

Methods: We studied 233 febrile children aged 3 months to 18 years presenting at the emergency department. Infection etiology (78 bacterial, 155 viral) was determined by clinical adjudication of three physicians and microbiological confirmation of pathogenic viral strains using multiplex-PCR applied to nasal swabs (Seeplex-RV15). Based on the manufacturer’s pre-determined cut-offs, ImmunoXpert generated one of three results: viral (score 0-35), equivocal (score 35-65) or bacterial (score 65-100).

Results: ImmunoXpert correctly classified 90% of bacterial cases and 91% of viral cases, when compared to the expert panel diagnoses (13% of patients had an equivocal result; Figure 1). For coronavirus, bocavirus, human metapneumovirus, and enterovirus, the assay classified all patients correctly. In the case of adenovirus, which is known to trigger a bacterial-like inflammatory host response, the assay correctly classified 83% of the patients. In comparison, CRP (cut-off: 40 mg/l) correctly classified only 42% of adenovirus infections.

Conclusion: The host-immune based assay represents a promising new tool for aiding clinicians in determining infection etiology in febrile children. Importantly, it may assist in distinguishing between adenovirus and bacterial infections, which can be associated with similar clinical presentation.

B-068

A Fully Automated Immunoassay for the Detection of Zika Virus Immunoglobulin M

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Objective: Zika virus is a mosquito-borne flavivirus in the family Flaviviridae and is closely related to dengue, yellow fever, and West Nile viruses. It is primarily transmitted by Aedes mosquitoes, which are found throughout the tropical and subtropical regions of over 100 countries.

During the first one to two weeks after onset of symptoms, Zika virus disease can be diagnosed by performing reverse transcriptase-polymerase chain reaction (RT-PCR) in the blood of symptomatic patients. Virus-specific IgM and neutralizing antibodies are typically present after the first week of illness and may be detectable for up to 12 weeks. Combined with patient demography and clinical findings, detection of IgM antibodies to Zika virus provides an essential tool for diagnosis and follow-up care of an acute or recent infection. The objective of this study is the development and validation of a rapid, reliable and accurate automated immunoassay for the detection of Zika virus IgM antibodies in human sera.

Methods: The LIAISON® XL Zika Capture IgM immunoassay is a chemiluminescent (CLIA) in vitro diagnostic designed for the LIAISON® XL automated analyzer platform, and is intended for the qualitative detection of Zika virus IgM antibodies in human sera. It is a two-step, antibody capture, sandwich assay that utilizes paramagnetic particles coated with monoclonal anti-immunoglobulin antibodies, followed by recombinant Zika virus non-structural protein 1 (NS1), labeled with a lumino-derived reporter molecule.

Validation: The LIAISON® XL Zika Capture IgM assay has been validated for precision, interference, and cross reactivity as well as clinical sensitivity and specificity. Intra and total assay imprecision are <9% and <14% respectively. The assay was evaluated for interference from endogenous substances including hemoglobin, serum albumin, bilirubin, triglycerides, cholesterol, HAMA and rheumatoid factor.
**Infectious Disease**

(RF). None of these compounds interfered in the assay. The assay was tested for cross-reactivity to other related flaviviruses using samples that were positive for Dengue virus or West Nile Virus IgM, or from subjects who had been vaccinated for Yellow Fever. No cross-reactivity was detected.

Clinical specificity was evaluated using 220 apparently healthy donors and 32 pregnant donors collected in the United States and presumed negative for Zika virus infection. The LIAISON assay was negative in 251 of the 252 samples, for a clinical specificity of 99.6%.

Clinical sensitivity was evaluated using serially collected samples from 56 symptomatic subjects (including 15 pregnant subjects) from the Dominican Republic found to be initially PCR positive for Zika virus. All subjects were detected by the LIAISON® assay as Zika IgM positive by the first draw after 8 days post-onset of symptoms, with detection in some subjects as early as 4 days and as late as 83 days post-symptom onset.

Conclusions: DiaSorin’s new LIAISON® XL Zika Capture IgM immunoassay has excellent diagnostic sensitivity and specificity with a demonstrated lack of cross-reactivity to related flaviviruses. The LIAISON® XL Zika Capture IgM immunoassay is the newest member of the DiaSorin infectious disease assay panel, providing continued assistance to clinicians for accurate detection of infectious agents.

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**Comparison of qSOFA (quick SOFA) Score, Presepsin, Procalcitonin and Lactate for Severity Assessment and Mortality Prediction in Patients with Initial Sepsis**

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Background

The SOFA score is associated with an increased probability of mortality in sepsis. The Third International Consensus Definitions for Sepsis and Septic Shock defined the qSOFA score, which can be assessed at admission without laboratory tests.

Objective

To compare sepsis biomarkers with qSOFA for differentiation of sepsis, severe sepsis or septic shock and risk of mortality prediction.

Methods

66 Patients admitted with signs of sepsis were included. Severe sepsis and septic shock were defined according to current guidelines. qSOFA score was calculated from respiratory rate, GCS score and systolic blood pressure using the recommended thresholds. Presepsin (PSEP) and procalcitonin (PCT) were determined using the POC assay PATHFAST Presepsin, LSI Medience Corporation and the BRAHMS luminescence immunoassay.

Results

Discrimination between sepsis (n=30, mortality=6.6%) and severe sepsis or septic shock (n=36, mortality=36.1%) revealed AUC values of 0.621, 0.627, 0.731, 0.740 and 0.781 for lactate, PCT, qSOFA, PSEP and the combination qSOFA+PSEP, respectively. 15 patients died during hospitalization. AUC values of mortality prediction were 0.715, 0.758, 0.734, 0.740 and 0.803 for lactate, PCT, qSOFA, PSEP and qSOFA+PSEP, respectively. qSOFA scores ≥2 should identify greater risk of death or prolonged ICU stay. Discrimination between qSOFA <2 and ≥2 revealed AUC values of 0.756, 0.669 and 0.606 for PSEP, lactate and PCT.

Using the threshold ≥2 of qSOFA and ≥500 ng/L of PSEP, the combination qSOFA+PSEP detected 14 non-survivors (93%) and 33 (92%) patients of the high-risk group (n=36), whereas qSOFA alone detected only 10 non-survivors (67%) and 21 patients of the high-risk group (58%).

Conclusion

The results demonstrated that the qSOFA score is not a standalone criterion for risk stratification in sepsis at admission. Simultaneous assessment by combining qSOFA and PSEP improved the validity significantly. The POC assay PATHFAST Presepsin showed superior performance compared to lactate and PCT.

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**B-069**

**RIG-I Enhances IFN-α Response by Promoting Antiviral Proteins Expression in Patients with CHB**

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Background: Chronic hepatitis B (CHB) infection is a serious health problem affecting approximately 400 million individuals worldwide. The main purpose in CHB treatment is to suppress viral replication. IFN-α has various biological properties, such as immune modulation, antiviral response, and antiproliferative activity. However, IFN-α has many side-effects, such as bone marrow suppression, influenza-like symptoms, and exacerbation of autoimmune illnesses. Moreover, only approximately 30% of HBV infected patients respond during interferon therapy. Thus, identification of molecular biomarkers to predict IFN-α therapy sensitivity would be useful in the clinic. In this study, we aim to clarify the mechanism of RIG-I in prediction of CHB therapy with IFN-α. Methods: A total of 65 CHB patients were recruited from The 1st Affiliated Hospital of Fujian Medical University and Liver Disease Center of Fujian Province between July 2013 and August 2015. All patients with CHB were treated with PegIFN weekly for 48 weeks and followed-up for another 24 weeks. Non-response is defined as less than 1 log10 IU/mL decline in serum HBV DNA level from baseline at three months of therapy or serum HBV DNA level > 2,000 IU/mL at the end of 1 year. Response is defined as serum HBV DNA level < 2,000 IU/mL and ALT normalization. Serum levels of HBV DNA, HBsAg levels and presence of HBsAg and anti-HBe were measured using an immunosorbent assay. Real-time PCR primer sequences for human RIG-I and antiviral proteins were obtained from the online NCBI public resource. Total RNAs from each sample in peripheral blood mononuclear cells (PBMCs) were extracted with use of a TRizol kit. Group measures were shown as mean ± SEM. A Student’s t test was used to analyze the differences between the groups. The p values were calculated in SPSS 18.0. and the statistical significance level was accepted as p < 0.05. Results: In this study, we found RIG-I expression was higher in responders than non-responders of CHB patients with IFN-α therapy. Compared with traditional clinical tests such as HBV DNA and HBsAg, RIG-I had more sensitivity and specificity in predicting IFN-α therapeutic response in CHB patients. Mechanistically, RIG-I enhanced IFN-α response by promoting anti-HBV proteins expression such as double-stranded RNA-dependent protein kinase (PKR), Oligoadenylate synthetase (OAS), adenosine deaminase (ADAR1) and Mx protein. Knocking out of RIG-I could downregulate the expression of these proteins above. Moreover, in HBV-transfected HepG2 (PHY106-HBV-HepG2) cell, inhibited RIG-I expression by RIG-I siRNA deceased STAT1 phosphorylation. Conclusion: Our results revealed RIG-I enhanced IFN-α response by promoting antiviral proteins expression via STAT1 pathway. RIG-I may be a new predictive factor for prediction of IFN-α efficacy in chronic hepatitis B patients.

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**B-072**

**Automated and laboratory information system integrated workflow for simultaneous detection of Zika, chikungunya and dengue viruses by RT-qPCR in EDTA-plasma, urine and seminal-plasma: a unique and comprehensive test routine for Brazilian arboviral threats.**


Background: The simultaneous detection of Zika (ZIKV), chikungunya (CHIKV) and dengue (DENV) viruses provide a comprehensive diagnostic answer for patients. RT-qPCR is the most reliable method to detect and distinguish these three viruses. The assay is mostly performed on blood substrates, the limitations are that the viruses RNA last for a few days and the absence of a high-throughput process (majority of RT-
Infectious Disease

IgM

3,227

9,226

5,275

Tuesday, August 1, 9:30 am – 5:00 pm

determined by testing CMV IgM-positive samples was ≥96%. Negative percent

Results:

evaluated per CLSI EP5-A3 by testing six samples with Index values spanning

Methods:

The assay validation enrolled negative EDTA-plasma, urine and seminal-plasma

pools/samples spiked with known amounts of each virus. The RT-qPCR workflow

was provided by the Flow classic solution (Roche), which accepts different matrices

simultaneously. Nucleic acids were extracted from 500ul of each matrix (seminal-

plasma was diluted 1:1 in PBS) by using viral NA universal LV 3.1 protocol. An

in-vitro transcribed randomized RNA sequence was added into samples during the nucleic

acids extraction and was co-amplified in all instances to function as a process control.

Primers/probes were obtained from literature. ZIKV, CHIKV and DENV were assessed

simultaneously, but in independent reaction wells. To determine the limits of detection

(LODs), 1:1 dilutions of each virus were tested (from 1000 to 0.98 copies/mL), the

LODs were calculated by probit regression analysis. The imprecisions near the

clinical decision point (grey zones) were assessed by repeating the LOD experiment in

triplicate during 5 days. The viral load ranges that did not allow certainty about the

infection status were determined in each matrix. The accuracies were evaluated by a

recovery strategy. The following numbers of samples were prepared for all tested specimens: ZIKV n=86 (21 positive), CHIKV n=85 (27 positive) and DENV n=84 (23 positive). Viral loads in positive samples ranged from 1x10^5 to 1x10^6 copies/mL in EDTA-plasma and urine and from 2x10^3 to 1x10^5 copies/mL in seminal-plasma. The agreements between the obtained and the expected results were evaluated.

Results:

The LODs in EDTA-plasma, urine and seminal-plasma, respectively, were 90.5 (95%CI 58-299), 51.6 (95%CI 40-74) and 136.3 (95%CI 111-183) copies/mL for ZIKV; 172 (95%CI 135-245), 112.6 (95%CI 92-150) and 551.8 (95%CI 451-730) copies/mL for CHIKV and 94.3 (95%CI 65-217), 38.8 (95%CI 31-53) and 145.6 (95%CI 1205-2023) copies/mL for DENV. The grey zones in EDTA-plasma, urine and seminal-plasma, respectively, were 250-7.8, 62.5-3.9, 250-7.8 copies/mL for ZIKV; 250-3.9, 250-7.8, 1000-31.3 copies/mL for CHIKV; and 125-7.8, 62.5-3.9, 2000-250 copies/mL for DENV. In the accuracy assay, the comparison between obtained and expected results revealed total agreement of 100% (95%CI 95-100%), positive agreement of 100% (95%CI 85-100%) and negative agreement of 100% (95%CI 94-100%) for all tested specimens. No cross-reaction was observed.

Conclusion:

The proposed workflow showed acceptable sensitivity, precision and accuracy for ZIKV, CHIKV and DENV detection in multiple body fluids allowing a unique and comprehensive test routine for Brazilian current arboviral threats.

B-074

Sero logical and molecular diagnosis of arboviruses in Brazil,2016

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BACKGROUND: Since the introduction of Zika (ZKV) and Chikungunya (Chkv) viruses to Brazil in 2014, several regions are witnessing the co-circulation of these two agents in addition to Dengue (Den) viruses which are endemic. Specific diagnosis of these pathogens is hampered by the cross-reactivity between flaviviruses (Denv and Zkv) and by shared symptoms. Although there is, as yet, no specific antiviral drug, patients shall be managed differently, according to the infectious agent. DASA is the largest Brazilian clinical pathology lab, covering a great extension of the nation, thus, reflecting on-going trends in the epidemiology of these 3 arboviral diseases.

METHODS: In year 2016, thousands of samples (table) were submitted to serological (IgG and IgM) of molecular testing upon medical request. Results were compiled and analyzed.

RESULTS: 57% (IgG) and 49% (IgM) of the samples submitted to Chkv serology were found reactive, for dengue 40% (IgG) and 23% (IgM) had positive results while these rates were much lower for Zika; 28% (IgG) and 3% (IgM). Among requests for ZKV and ChKV, women were approximately 70% of the patients, while for Deng they were 55%. Chkv, Den and ZKV RNA were detectable in 18%, 7% and 5% of the samples respectively. ZKV RNA positivity was maximum in January (13%) declining over time.

CONCLUSIONS: The clinical hypothesis of Chkv had the highest rate of laboratory confirmation, both serological and molecular, probably reflecting the typical arthralgia associated to this infection. Low positivity for Zika tested samples may be attributed to a large number of symptomless patients being submitted to testing for reproductive planning, since this rate was similar among both genders. The highest rate of viremic samples in January suggests that in 2016 Zika outbreak happened earlier than observed for Dengue, which usually peaks in March/April.

Table – Number of samples submitted to arbovirus testing at DASA, Brazil, 2016

<table>
<thead>
<tr>
<th>MARKER</th>
<th>IgG</th>
<th>IgM</th>
<th>RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHKV</td>
<td>33,762</td>
<td>33,651</td>
<td>382</td>
</tr>
<tr>
<td>DENV</td>
<td>18,875</td>
<td>21,825</td>
<td>150</td>
</tr>
<tr>
<td>ZKV</td>
<td>5,275</td>
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</table>

B-075

Improvement to Workflow in Reagent Dispense System Design in the New Beckman Coulter DxM MicroScan WalkAway System


The DxM MicroScan WalkAway instrument system is used with MicroScan panels for identification of microorganisms and detection of their susceptibility for relevant antimicrobial agents. In an effort to enhance user workflow, the DxM MicroScan WalkAway instrument reagent dispense system was re-designed to include a more robust liquid level sensing technology paired with a unique indicator for each reagent, oil, and waste container. The indicator lights are visible on the instrument exterior without having to interrupt instrument processing, improving workflow and usability. The bottle cap assembly employs a quick release mechanism that disconnects the reagent bottle and cap from the tubing allowing the operator to more easily replace reagents according to laboratory safety protocols. In conclusion, Beckman Coulter’s
Infectious Disease

Results:

Of the 110 samples genotyped by the microarray base test, the concordance rate was 93.42% (71/76) for genotype 1, 100% (15/15) for genotype 2, 85.71% (12/14) for genotype 3 and 100% (5/5) for HCV negative samples. While no HCV genotypes 4, 5 or 6 were identified by either test, these genotypes are known to be rare in North America. Overall, the concordance rate was 93.64% (103/110) for all samples tested.

Conclusions:

This preliminary study of an HCV single-tube, one-step RT-PCR, microarray based test shows a good correlation with the Siemens Versant HCV Genotype 2.0 Assay. The microarray based test runs on an automated system and provides for reduced hands-on time.

FOR RESEARCH USE ONLY

B-078

Exploring Potential Quasispecies Variants of Influenza H1N1pdm09 Virus

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Background and Objectives: Swine-origin H1N1 virus, also known as H1N1pdm09, emerged in 2009. Studies in 2011 revealed that a cooperative interaction of two genetic variants, namely viral quasispecies, increased population-level fitness and promoted viral growth, e.g., Asp (D) to Gly (G) at position 222 in the hemagglutinin gene (HA-222). Incidence of H1N1pdm09 has risen to reach a new high in 2015. An outbreak in Taiwan caused over 1400 severe cases and 110 deaths from July 2015 to March 2016. However, little is known about the association between quasispecies variants and disease severity in this outbreak. Two objectives in this study were to detect genomic variants as potential signatures of viral quasispecies, in particular for 2015-16 outbreak strains, and to further estimate the prevalence of quasispecies variants from 2009 to 2016.

Methods and Results: We provided 10 Taiwanese H1N1pdm09 genomes isolated in the 2015-16 season by Sanger sequencing, including five severe (four patients had pneumonia, pleural effusion, and adult respiratory distress syndrome, and one had pneumonia and myocarditis), and five non-severe (four had upper respiratory infections, and one pneumonia case without other complications) cases. Influenza genome in approximately 13.5 Kb contained eight segments which included PB2, PB1, PA, HA, NP, NA, MP, and NS. In the 10 Taiwanese genomes, NA-74, -151, -314, and NS2-22 were detected, exhibiting more diversity than other positions. Only NA-151 showed residue “X” (as codon “RAC”) and “D” (“GAC”) in all 5 severe and 5 non-severe cases perfectly. This nucleotide ambiguity code “R” presented a dominant variant as “G” and a minor as “A”. To estimate the prevalence of this minor variant, 7469 H1N1pdm09 genomes from 2009 to 2016 were downloaded from GISAID database. 94 translated nucleotide sequences exhibited non-D residues at NA-151, including 89”X”, 4 “E”, and 1 “N”. Interestingly, increasing counts of X-residue in database. 94 translated nucleotide sequences exhibited non-D residues at NA-151, including 89”X”, 4 “E”, and 1 “N”. Interestingly, increasing counts of X-residue in virus.
Performance evaluation of Lumipulse HTLV-I/II


Background: HTLV (Human T-cell lymphotropic virus) infects a type of white blood cell called T-cell or T-lymphocyte. There are two types HTLV, HTLV-I and HTLV-II, closely related human C retroviruses. HTLV-I is endemic in the Caribbean, Japan, South America, and parts of Africa. HTLV-II has been recognized as a cause of adult T-cell leukemia (ATL), HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP). HTLV-II is found among Native Americans and injections drug users in many city of Western Europe and North America. We have developed Lumipulse G HTLV-I/II as screening kit which can detect both anti-HTLV-I and anti-HTLV-II antibodies. This report presents the performance evaluation of Lumipulse G HTLV-I/II.

Reagent: Lumipulse G HTLV-I/II is an assay system for the qualitative detection of anti-HTLV-I in specimens based on CLEIA technology by a two-step sandwich immunoassay method on the LUMIPULSE G System. This reagent uses a recombinant protein and peptides derived from three env proteins (HTLV-I gp21, HTLV-I gp46, HTLV-II gp46) and two gag proteins (HTLV-I p19, HTLV-II p19). The two step sandwich assay method is used to detect antibodies against these antigens. The amount of anti-HTLV antibodies in a specimen is automatically calculated from the calibration data. The result of the calculation is reported in cutoff index (C.O.I.) and then it is interpreted if the specimen is “reactive (C.O.I. ≥ 1.0)” or “non-reactive (C.O.I. < 1.0)”.

Methods and Results: Precision: The four negative specimens, the five positive serum samples and the four positive plasma samples were tested in duplicate per run, two runs per day for 20 test days, total 80 measurements per sample. The proportion of concordant test results was 100.0 % for each sample tested. For the positive samples, the within-run %CV ranged from 1% to 2%, the total %CV ranged from 2% to 3%. Specificity: A total of 5939 anti-HTLV-I/II negative samples were tested. These samples had negative result with Abbot Architect HTLV and included 230 potentially interfering samples. The results were non-reactive for 5933 samples, demonstrating a specificity of 99.9%. Sensitivity: A total of 300 anti-HTLV-I and 100 anti-HTLV-II specimens from different HTLV patients were tested. These samples had positive result with Abbot Architect HTLV. The results of Lumipulse G HTLV-I/II were reactive for 400 samples, demonstrating a diagnostic specificity of 100.0%. Dilution sensitivity: The diluted 20 positive samples were tested by Lumipulse G HTLV-I/II and Abbot Architect HTLV. Lumipulse G HTLV-I/II had equivalent or better dilution sensitivity compared with Abbot Architect HTLV.

Conclusion: Lumipulse G HTLV-I/II was developed by Fujirebio,Inc which can detect the antibodies against the three env proteins (HTLV-I gp21, HTLV-I gp46 and HTLV-II gp46) and the two gag proteins (HTLV-I p19 and HTLV-II p19) simultaneously. It was indicated that Lumipulse G HTLV-I/II has sufficient performance as HTLV screening kit.

Evaluation of Biochemical and Hematological Markers of Cerebrospinal Fluid in Suspected Patients with Meningitis

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Background: The cerebrospinal fluid (CSF) is a fluid that frequently received by the laboratory to analyze it for different parameters to aid physicians in diagnosis of many diseases. One of particular disease that required CSF fluids is Meningitis. The CSF and blood culture are gold standard test in establishing the diagnosis of Meningitis, however, it is labor intensive and require longer turn-around time before the information is amiable to the physician. There were a lot of effort to explore a better and quick markers in CSF to assist in rapid assessment for Meningitis. The aim of this study was to evaluate the levels of biochemical and hematological markers in the CSF samples received in our laboratory and compare them with the gold standard CSF culture. Methods: A total of 248 CSF samples were received from 137 patients, who were admitted to our hospital suspected of having Meningitis. The samples were collected by our physicians and sent to the laboratory to be analyzed immediately. Hematological and biochemical markers were performed in these samples including white cells (WC), glucose (Glu), and total protein (TP). The analyzers used in this study were Advia 2120 from Siemens Company for hematology markers and Architect from Abbot Company for biochemical markers. CSF culture were done for all samples in the microbiology laboratory. Statistics were done using SPSS IBM software version 20. The diagnostic sensitivity, and specificity were calculated. The p value of < 0.05 were chosen to be significant. Results: The majority of samples were received from neonate (<1 year) patients 61 (44.5%), children (<18 years) patients 36 (24.3%) and adult (>18 years) patients 40 (29.2%). Female were 47 (34.3%). The culture were positive in 195 CSF samples, 180 (92.3%) were negative and 15 (7.7%) were found positive for bacterial meningitis. The sensitivity for WC, TP and Glu were found to be 73.3%, 86.7%, and 60% respectively, while the specificity were found to be 75.4%, 36.1%, and 30.7% respectively. The positive predictive value (PPV) were calculated 20%, 10.2%, and 6.7% respectively, while the negative predictive value (NPV) were found to be 97.1%, 97%, and 90.2% respectively. The overall accuracy of these tests were found to be 75.3%, 40% and 33% respectively. Conclusion: These tests showed a good sensitivity but low specificity with exception of WC. These tests were good enough to rule-out meningitis.
Clinical Performance of Elecsys® HBsAg II in Subjects with Increased Risk of Hepatitis

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A multicenter clinical performance study of Elecsys® HBsAg II immunosassay and Elecsys HBsAg confirmatory test on cobas e 601 analyzer was recently completed at three US sites. Study population consisted of adult, pregnant and pediatric subjects at risk for hepatitis (sexual practice, behavior, medical status or occupation). Elecsys assay is an automated sandwich immunosassay based on the chemiluminescence principle where complexes of sample HBs antigen, biotinylated/ruthenylated-anti-HBsAg antibodies, and streptavidin-magnetic microparticles are captured on an electrode. The primary objective was to evaluate percent agreement between Elecsys HBsAg II and reference assay. The secondary objectives included evaluation of specificity, seroconversion sensitivity, and imprecision.

Abbott ARCHITECT HBsAg and HBsAb confirmatory reference testing was performed at two US sites. Final HBsAg interpretation was based on the confirmatory testing of repeatedly reactive samples. Positive/negative percent agreements with 95% confidence limits for various cohorts are listed below.

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Percent Agreement, CL</td>
</tr>
<tr>
<td>Adult</td>
<td>39</td>
<td>100.00%-90.97-100.00%</td>
</tr>
<tr>
<td>Supplemental</td>
<td>391</td>
<td>99.23%-99.77-99.84%</td>
</tr>
<tr>
<td>Pediatric</td>
<td>0</td>
<td>NA NA</td>
</tr>
<tr>
<td>Pregnant</td>
<td>13</td>
<td>100.00%-75.29-100.00%</td>
</tr>
</tbody>
</table>

1Population to prove prevalence of acute and chronic hepatitis B subjects

Evaluation of Elecsys HBsAg II assay and Elecsys HBsAg confirmatory test on cobas e 601 analyzer demonstrated acceptable clinical/analytical comparison against the reference assay.

Evaluation of the Utility of CMS Claim Data for Early Detection of Increasing Influenza Activity

L. Fan1, R. Astles2, H. Burkholm2. 1Centers for Disease Control and Prevention, Atlanta, GA, 2Johns Hopkins University, Applied Physics Laboratory, Baltimore, MD

Objective: To evaluate the utility of CMS Medicare claim data for early detection of increasing influenza (flu) activity using weekly volume of flu diagnoses (V_dx) or flu rapid tests (V_test) irrespective of the test results, which are not included in CMS data.

Methods: We compared CMS Medicare outpatient claims (OTP) data with positive test volumes using rapid flu tests from the CDC National Respiratory and Enteric Virus Surveillance System (NREVSS) as the “gold standard” for flu activity. We compared both V_dx and V_test to the gold standard for each of 10 selected states (one per HHS region). Spline models were developed and tested to identify the weeks of yearly volumes of V_dx, V_test, and NREVSS test positives (dependent variables) for 5 influenza seasons (2007 to 2012), with the week number as the independent variable. Using the fitted models, we calculated predicted weekly volumes and standard deviations (SD) for V_dx and V_test. Criteria for an initial alert were defined as: 1. an increase of the predicted volume from the previous week by two SD (2 SD criterion), or 2. an increase of the predicted volume from previous week by 0.45 SD for two consecutive weeks (0.45 SD criterion). A flu “episode” was defined as the interval between an initial alert and a drop in volume meeting the same criterion.

Results: We detected 67 flu episodes from the 10 states in NREVSS (2007-12) using spline models. With the 0.45 SD and 2 SD criteria respectively, the OTP predictors using V_dx data identified 60 and 41 out of the 67 episodes, and 64 and 49 episodes out of the 67 episodes using V_test data. With the 0.45 SD criterion, 83% of episodes detected using V_dx and 85% using V_test were also detected in NREVSS. Using the 2 SD criterion, 93% of episodes detected using V_dx and 98% using V_test were also detected in NREVSS.

With the 0.45 SD criterion, V_dx (V_test) detected flu activity an average of 3.1 (5.7) weeks earlier than NREVSS predictions. With the 2 SD criterion, V_dx (V_test) detected flu activity an average of 3.6 (2.6) weeks later than NREVSS predictions.

Conclusions: This study demonstrated the potential use of CMS claims data for early detection of increasing influenza activity in specific regions of the U.S. In this retrospective analysis, spline models performed well for early detection of an increase in flu activity. The 0.45 SD criterion demonstrated greater sensitivity over the 2 SD criterion yielding earlier detection by an average of over three weeks than predictions.
B-086
Performance Evaluation of the ROCHE E 170 for the Determination of Procalcitonin in blood.

Background: Sepsis is a systemic inflammation response caused by infection. The rates of hospital admissions for sepsis exceed those of myocardial infarction and stroke and it is a major health care expense due to the high incidence and mortality. Early intervention prevents the sepsis to progress to sepsis and septic shock. The dilemma begins with proving the presence of infection and the need for a reliable marker to diagnose sepsis; however laboratory tests for sepsis were either non-specific or require longer turn-around time. Procalcitonin could be the promising biomarker; it is precursor for calcitonin and secreted from C-cell in the thyroid but under certain circumstance like systemic infection procalcitonin is secreted from other cells making its concentration rises in the blood. Studies have shown that its concentration in the blood increase rapidly in patient with sepsis; in addition, its concentration correlate well with the severity of the infection, the higher concentration of procalcitonin are associated with higher risk to progress to septic shock.

Methodology: Roche Elecsys BRAHMS procalcitonin assay is a sandwich assay with a total incubation of 18 minutes. The assay is electrochemiluminescence immunoassay “ECLIA” with 2 incubations; results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode. We evaluated: the sensitivity, linearity, within assay coefficient variation for replicates from synthetic materials and from patient sample, between assay coefficient of variation, reportable range, and correlation was done using reference laboratory. Statistical analyses were done using Analyse-it.

Results: the sensitivity was 0.02 ng/mL using non-serum matrix sample and 0.06 ng/mL for human sample. Within assay coefficient variations were 9.2%, 2.0%, and 1.0% for a concentration of 0.021 ng/mL, 0.462 ng/mL, and 9.367 ng/mL respectively. Between assay coefficient variations were 4.7% and 6.5% for a concentration 0.47 ng/mL and 9.0 ng/mL respectively. Analytical range was verified from 0.02-100 ng/mL. Regression analysis between the reference laboratory and Roche PCT gave a slope of 1.02 and intercept 0.01 and correlation coefficient of 0.9738.

Conclusion: the Roche Elecsys BRAHMS procalcitonin assay gave the benefit of a fully automated, high throughput, high precision and acceptable sensitivity assay. Although the sensitivity of the assay was above the published cut-off for healthy people but it was well below the range for systemic bacterial infection. The accuracy, precision, and sensitivity of the assay make it suitable as a diagnostic marker and a part of the antibiotic stewardship by monitoring the progression of infection and when to begin or stop the antibiotics.

B-088
Burkholderia Cepacia Outbreak in Long-Term Care Facilities.

Background: Burkholderia Cepacia, a gram negative bacilli, is a group or complex of bacteria that can be found in soil and water. It is more colonizing bacteria than infecting bacteria; however it may cause problem if found in people with weak immune system, cystic fibrosis, or if found in sterile body fluid. A recent multistate outbreak of Burkholderia Cepacia in blood was reported due to contaminated prefilled saline flush was reported. A majority of the cases are reported in long-term care facilities or rehabilitation facilities. Every set included two vials (aerobic and anaerobic) which were incubated in a Bactec instrument. Positive cultures were subcultured and then identified using Microscan96 Walkaway conventional panels. Data analyses were done for all the facilities and then isolating the facilities with positive isolate for Burkholderia Cepacia. Statistical analyses were done using Analyse-it.

Results: 15.4% of the total blood cultures were positive. Burkholderia Cepacia accounted for 12.8% of these positive cultures, and was found only in 7 facilities, accounting for 127 of the 1276 cultures tested. Of these 127 patients, 28% of them had a positive blood culture. Of those positive cultures 71.4% of the bloodstream infections were due to Burkholderia Cepacia. No deaths to our knowledge were reported due to Burkholderia Cepacia in the identified patients.

<table>
<thead>
<tr>
<th>% positive blood culture</th>
<th>All Facilities</th>
<th>Affected facilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>% B. Cepacia/all blood culture</td>
<td>2.0%</td>
<td>19.7%</td>
</tr>
<tr>
<td>% B. Cepacia/total positive blood culture</td>
<td>12.8%</td>
<td>71.4%</td>
</tr>
</tbody>
</table>

Conclusion: Burkholderia Cepacia is a threat if found in blood culture, especially in long-term care facilities where most of the residents are elderly, frail, disabled, and are on multiple medications; in addition, Burkholderia Cepacia is resistant to common antibiotics. Early detection and appropriate treatment would benefit the patient. Also the awareness of the contaminated saline decreased the spread of the bacteria to other patients.

B-089
Performance Evaluation of the VERSANT HCV Genotype 2.0 Assay (LiPA)

Background: Determination of the HCV genotype is important to prescribe appropriate HCV treatment and predict response to antiviral treatment. HCV genotyping provides clinicians with important, useful tools to optimize HCV treatment type, dose, and duration, allowing the best chance of eradicating HCV from the patient’s body.

Methods: The VERSANT HCV Genotype 2.0 assay (LiPA) is a line probe assay that identifies HCV genotypes 1-6 and subtypes a and b of genotype 1 in human serum or plasma specimens. Using reverse hybridization technology, biotinylated DNA PCR product generated by RT-PCR amplification of the 5’ UTR and core regions of HCV, RNA is hybridized to immobilized oligonucleotide probes. Analytical validity and clinical validity studies were performed at three clinical trial sites to validate the performance of the VERSANT HCV Genotype 2.0 assay (LiPA). The genotyping rate (GR) and genotyping accuracy (GA) were evaluated for each HCV genotype/subtype in order to assess the integrity of the assay results compared to the NSSb reference method. Results were interpreted manually and using the semi-automated method.

The clinical utility of the VERSANT HCV Genotype 2.0 assay (LiPA) was assessed by evaluating the association between HCV genotype and the probability of achieving SVR, defined as undetectable HCV RNA levels (<25 IU/mL) 12 weeks after cessation of treatment (SVR12) using clinical samples treated with one of the following combinations: a) sofosbuvir (SOF), pegylated interferon (peg-IFN), and ribavirin (RBV); b) SOF and RBV; or c) SOF and ledipasvir (LDV).

Results: The results for the analytical validity study are as follows: The GR for each HCV genotype/subtype ranged from 86.7 to 100% for the manual interpretation method and 83.3 to 100% using the semi-automated method. The GA for each HCV genotype/subtype using either method of interpretation was 100% except for subtype 1a (99%) and 1b (98%).

In the clinical validity study, the SVR12 rate for all patients tested was 88.5% (192/217) for both methods. For individual genotypes/subtypes, the observed SVR12 rates ranged from 72.5 to 100%. Results indicate patients diagnosed with genotype 1 had significantly lower SVR12 rate compared to pooled non-1 genotype patients. The relationship between the HCV genotype/subtype and the SVR12 rate is essential for achieving successful clinical outcomes in chronically infected HCV patients treated with direct-acting antiviral (DAA) regimens.

Conclusions: The VERSANT HCV Genotype 2.0 assay (LiPA) provides interpretable and accurate diagnosis of HCV genotypes 2, 3, 4, 5, and 6 and subtypes 1a and 1b as shown by an overall GR and GA rate of ≥88% and ≥99%, respectively, in both studies. SVR12 rate by genotype/subtype is consistent with published results using similar treatments with DAAs.

The clinical evaluation of the assay demonstrates reliable identification of HCV genotype/subtype for optimal patient therapy.
Infectious Disease

VERSANT® HCV Genotype 2.0 Assay (LiPA) [Reagents: Genotype 2.0 Kit, Amplification 2.0 Kit, and Control 2.0 Kit] is CE-marked in Europe. For Research Use Only in the United States. Product availability varies from country to country and is subject to local regulatory requirements.

**B-090**

Automated Molecular Detection of Helicobacter pylori and its Resistance to Clarithromycin in Human Tissue Biopsies

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Introduction:
According to the World Health Organization (WHO), Helicobacter pylori is a class 1 carcinogen that affects approximately 50% of the world’s population. H. pylori in the gastric mucosa can lead to gastritis, duodenal ulcers and gastric cancer. When an endoscopy is performed in patients with dyspepsia, a gastric biopsy should be taken to evaluate for the presence of H. pylori. Infected patients should be offered some form of eradication therapy. The eradication therapy involves the use of a combination of antibiotics, such as amoxicillin, metronidazole, clarithromycin, combined with proton pump inhibitors; however, antibiotic resistance is a major cause for treatment failure. The gold standard diagnostic procedure for H. pylori detection is the use of a gastric fluid culture and antimicrobial susceptibility testing which typically takes a minimum of 5 days to obtain a definitive result. Other more rapid methods have low sensitivity for the detection of H. pylori including the helicobacter-urease assay from gastric biopsies, antigen testing from stool or breath tests.

Objective:
The objective of our study was to evaluate a molecular based diagnostic test for H. pylori called the Hpylori-Q Assay (Autogenomics, Inc., Carlsbad, CA), an automated multiplexed film-based microarray assay that can simultaneously detect H. pylori and its resistance to clarithromycin from human biopsy samples in a clinic setting.

Results:
A total of 100 gastric biopsies were performed to isolate genomic DNA (50 H. Pylori positive and 50 H. Pylori negative), from specimens previously evaluated with the M-PCR assay (Tsang et al, Gastroenterol Res Pract. 2012). The 100 DNA samples were retested with the Autogenomics Hpylori-Q assay. The results showed that, the Hpylori-Q assay detected 48 out of the 50 H. pylori positive DNA specimens (96% sensitivity) and identified all of the 50 negative DNA as negative for H. pylori (100% specificity). The positive predictive value was 100% with a negative predictive value of 96%. In addition, 17 out of the 50 positive specimens were tested as clarithromycin resistant (34%), while 9 had a A2142G point mutation, another 9 DNA carried A2143G point mutation and one carried both A2142G, and A2143G mutations. The limit of detection for H. pylori was 22 copies per test for the Hpylori-Q assay.

Conclusions:
In this study, we demonstrated that the Hpylori-Q Assay, a molecular based DNA microarray assay is not only extremely sensitive for detecting H. pylori but highly specific for H. pylori and can identify specific clarithromycin resistant strains to better manage treatment of H. pylori. The Hpylori-Q Assay can facilitate the appropriate selection of treatment for patients with H. pylori infection that have clarithromycin resistance.

**B-091**

Panel of Zika virus infection diagnosis.


Background. Laboratory diagnosis of Zika virus (ZIKV) infection depends on the period of infection. During viremic period, diagnosis is based on PCR; and during nonviremic period, over one week from the clinical onset, is based on serology - IgM detectable for 3 to 6 months, and IgG probably lifetime. ZIKV-PCR is detectable on blood within the first week of clinical illness and on the urine up to 21 days after infection performed at the laboratory during 2016. ZIKV-PCR is detectable for 3 to 6 months, and IgG probably lifetime. ZIKV-PCR is detectable on blood within the first week of clinical illness and on the urine up to 21 days after infection performed at the laboratory during 2016.

**Methods.** During 2016 we performed 30,799 tests for ZIKV diagnosis; 78 samples were submitted to the full panel of diagnosis, defined as RT-PCR (on blood and/or urine) and ELISA serology performed at the same blood collection.

The serology (Eurimmun kit) is defined as negative (index <0.8), indeterminate (index range from 0.8 to 1.09) and positive (index >1.09).

**Results**
Majority of patients were women (60; 78%), average age was 35.8. Seventy-three patients were non-reactive by PCR from blood (table 1). Only one sample presented positive PCR on urine. At the time of panel sample collection, 18 samples (23%) were IgG positive.

<table>
<thead>
<tr>
<th>PCR urine</th>
<th>ELISA IgM</th>
<th>ELISA IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>Indet/ Negative</td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>67</td>
</tr>
<tr>
<td>60</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>98.7%</td>
<td>76.9%</td>
<td>50.7%</td>
</tr>
<tr>
<td>Not done</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>72</td>
<td>23.1%</td>
</tr>
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<td>92.3%</td>
<td>76.9%</td>
<td>60</td>
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<tr>
<td>60</td>
<td>45</td>
<td>30</td>
</tr>
</tbody>
</table>

Conclusions. Our results demonstrated that concomitant PCR and serology add no value to the laboratory diagnosis. It is important to optimize the laboratory investigation of ZIKV infection to have the highest diagnostic yield and save costs; serology testing should be performed for samples not tested by PCR or those are found to be negative. IgG positivity may be partially attributed to cross-reaction with antibodies due previous flavivirus infection.

**B-092**

Frequency of instrument, environment, and laboratory technologist contamination during routine diagnostic testing of infectious specimens

M. L. Yarbrough, J. H. Kwon, M. A. Wallace, T. Hink, A. Shupe, V. J. Fraser, C. D. Burnham. Washington University School of Medicine, Saint Louis, MO

Background. Laboratory testing to support the care of patients with highly infectious diseases such as Ebola virus may pose a risk for laboratory workers. However, data on the risk associated with various laboratory procedures and virus transmission during routine laboratory testing are sparse. Our objective was to evaluate contamination of laboratory equipments and the laboratory environment, and the laboratory worker during routine analysis of patient specimens using two approaches—fluorescent markers and a molecular surrogate of a high-titer viral infection. Methods. To mimic Ebola virus, which can be present in very high concentrations in blood and body fluids, de-identified remnant specimens were “spiked” with the MS2 bacteriophage, a single-stranded RNA virus that is nonpathogenic to humans, at a concentration of virus of 1.0 x 10^3 PFU/mL. The exterior of specimen containers was treated with a fluorescent Clo Germ powder (not visible to the eye) to visualize the contamination of laboratory surfaces during routine testing on point-of-care (POC) instruments. Laboratory testing performed and matrix tested included FilmArray Biothreat panel (BioFire, blood), FilmArray Gastrointestinal panel (BioFire, stool), FilmArray Respiratory Panel (BioFire, nasopharyngeal swab in transport medium), FilmArray Blood Culture Identification panel (BioFire, positive blood culture broth), Xpert Flu/RSV (Cepheid, nasopharyngeal swab in transport medium), urine dipstick (urine), BinaxNOW Malaria antigen (Abbott, blood), and a Piccolo comprehensive metabolic panel (Abaxis, plasma). Laboratory testing was performed by two experienced laboratory technologists using standard testing and cleaning procedures and with standard laboratory personal protective equipment (PPE) and procedures. Testing was performed on multiple days. After each test was setup, gloves were removed and samples were run on the corresponding POC instrument or test device. To assess for contamination, laboratory surfaces and the PPE and skin of laboratory technologists was monitored for transfer of fluorescent markers with UV light and the MS2 molecular marker using RT-PCR (Cepheid Smart Cyclers). Results: Transfer of fluorescence to gloves was observed during all rounds of routine testing. Fluorescence transfer to bare hands and contamination of the biosafety cabinet surface was observed in 6/16 (38%) and 7/16 (44%) tests performed, respectively. Fluorescence was observed on test cartridges/devices and auxiliary equipment/reagents in 4/14 (29%) and 8/12 (67%) tests performed. Importantly, no fluorescence transfer to downstream laboratory instrumentacion, hardware, or exposed surfaces was observed.

Conclusion: Simulation of grossly contaminated specimens using a fluorescent marker illustrated that proper adherence to PPE and procedures during testing setup prevented further contamination of the laboratory environment. These studies may increase our understanding of the risk for transfer of highly infectious samples to laboratory surfaces to better inform recommendations for laboratory safety practices and reduce the risk of pathogen transmission to laboratory workers.
Table 1. Mycobacteria species identified among 56 positive samples.

<table>
<thead>
<tr>
<th>Mycobacterium species</th>
<th>&lt;18 y</th>
<th>≥18 y</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium abscessus</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>3</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Mycobacterium kansasii</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>1</td>
<td>5</td>
<td>11</td>
</tr>
</tbody>
</table>

Conclusions. Among a total 56 clinical specimens submitted to Mycobacterial species identification, the majority (77%) was MNT, even among respiratory samples, demonstrating the relevance of additional identification of samples positive for mycobacteria.
Infectious Disease

Methods:

Syphilis screening is performed in our hospital using Architect CMIA, results with signal >1.0 are considered positive by the manufacturer. All results >1.0 are confirmed using TPPA. 2014 and 2015 results were collated and positive CMIA results were analysed in terms of sensitivity. CMIA and TPPA discordant results were analysed together with other laboratory and clinical results. For patients with repeat testing, the first result was analysed. Rapid Plasma reagin (RPR) was performed for TPPA positive cases.

Results:

470 (2.49%) results were >1.0. The median age for CMIA signals >1.0 and <1.0 were 44 and 35 years old, while their female/male ratio were 1.3 and 3.1 respectively. Out of 470 results with CMIA signal >1.0, 74 were non-reactive, 10 were indeterminate and 386 were reactive on TPPA, with 84% sensitivity. Their median CMIA was 1.62, 1.62 and 17.88, while their median age were 36, 64 and 47 years old respectively. Of 386 TPPA reactive results, 167 were RPR negative, and median RPR titre was 4 for RPR positive results.

The higher the signal value, the higher the sensitivity of CMIA results.

Conclusions:

In our population, there were a significant number of false positive results using Architect CMIA as first-line screening for syphilis followed by TPPA for confirmation. The sensitivity in this study is significantly lower than manufacturer report of >99.0% in package insert. Higher CMIA signals were associated with higher TPPA positivity and may be useful to predict true positivity.

B-097

Dengue diagnosis challenges in areas cocirculating other arboviruses: Brazilian dilemma situation.

L. C. Pierrotti, M. Guerino da Silva, G. Campana, A. do Nascimento. DASA Diagnósticos da América, Barueri, São Paulo, Brazil

Background. ZIK virus was first identified in Brazil in March 2015 and since than ZIK and DEN virus are cocirculating in the country. These two diseases cause similar symptoms (fever, headache, myalgia, arthralgia, and rash) requiring confirmatory diagnosis. Over one week from the onset of symptoms (nonviremic period) the diagnosis is based on serology, but the antibodies present cross-reactivity with related viruses of the same group.

Methods. DEN and ZIK serology of four Brazilian kidney transplant recipients who had diagnosis of DEN based on positive IgM from May 2014 to April 2015 were studied to check which virus are in fact related to symptoms.

The serologies were performed at least one year post-DEN diagnosis. DEN ELISA serology is defined as negative (index <0.9), indeterminate (index range from 0.9 to 1.1) and positive (index >1.1). ZIK ELISA serology is defined as negative (index <0.8), indeterminate (index range from 0.8 to 1.1) and positive (index >1.1).

Results. Characteristics of the cases are demonstrated in table 1. All but one presented negative IgG ZIK serology, confirming the previous diagnosis of DEN. However, in one case it was not possible to confirm the previous DEN diagnosis since both DEN and ZIK checked serology were positive.

Table 1. Description of DEN infection among four kidney transplant recipients.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Time from KT (days)</th>
<th>DEN diagnosis based on IgM</th>
<th>ZIK diagnosis based on IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>Male</td>
<td>1.7</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>Male</td>
<td>6.6</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
<td>Male</td>
<td>4.4</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>54</td>
<td>Female</td>
<td>3.0</td>
<td>0.5</td>
<td>2.7</td>
</tr>
</tbody>
</table>

Conclusions. Positive DEN and ZIK serology may represent prior exposure to flaviviruses or prior vaccination for Yellow Fever or Japanese Encephalitis. IgM antibodies is detectable 3 to 5 days and become undetectable in 2 to 3 months after exposure and IgG antibodies appear later and remain detectable for months. In areas of cocirculating these arboviruses it is important perform simultaneously the serology of DEN and ZIK to allow a more accurate flavivirus infection diagnosis. Transplant recipients are peculiar because they have an immunosuppression factor as an additional confounding factor.

B-098

Diagnosis of Clostridium difficile hypervirulent strain BI/NAP1/027 using Xpert C. difficile PCR assay in Brazil.

L. C. Pierrotti, L. ScarPELLi, P. Nishimura, L. Galindo, J. Levi, G. Campana, J. Alves, L. Faro. DASA Diagnósticos de América, Barueri, São Paulo, Brazil

Background. Clostridium difficile-associated disease (CDAD) is caused by spore-forming bacterium currently considered one of the most important healthcare-associated infection, being the main cause of hospital acquired diarrhea, associated with a high cost of hospitalizations and treatment. A hypervirulent C. difficile strain, denominated BI/NAP1/027, has caused outbreaks in North America and Europe but there are only a few reports of cases in Latin America.

Methods. We analyzed the Clostridium difficile toxin test in stool specimens collected from patients with suspicion of CDAD from Jan to Dec 2016, tested by Xpert C. difficile assay. The Xpert C. difficile PCR assay is a real-time PCR that detects the toxin B gene (tdcB), the binary toxin gene (tdc), and the tcdC gene deletion at nt 117. Assay includes reagents for the detection of Toxin producing C. difficile and toxin producing C. difficile 027/NAP1/B1.

Results. Throughout 2016 a total of 1,544 of CD toxin test were performed from 1,255 patients; 210 patients performed more than one test (average 1.2, range 2 - 6). The general positivity rate was 1.6% (253/1542) and 2% of samples had indeterminate results. A total of 10 patients presented BI/NAP1/027 positive strain.

Conclusions. CDAD caused by BI/NAP1/027 strain was detectable in few samples during 2016; however, laboratory methods that allow the identification of this hypervirulent strain are important both from the point of view of patient care, recognizing the possibility of infections with greater potential for severity, and epidemiologically, with active surveillance.

B-099

Performance evaluation of the new rapid AFIAS system to detect hepatitis C virus infection

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Background: The availability of accurate, rapid and cost-effective screening test for Hepatitis C virus (HCV) infection may be useful in smaller laboratories that cannot afford automated analyzer. This study explored the performance evaluation and diagnostic accuracy of newly developed AFIAS Anti-HCV assay (Boditech Med Inc. ChunCheon, Korea) which is an immune lateral flow cartridge test using small bench-top fluorescence reader for the detection of HCV antibody in serum and whole blood specimens in 20 minutes.

Methods. A total of 1,500 samples were used to compare AFIAS Anti-HCV assay with Elecsys anti-HCV II (Roche Diagnostics, Germany). The discrepant results were confirmed by recombinant immunoblot assay, Deciscan HCV Plus (Biorad). The HCV RNA-positive EDTA plasma specimens (SerCare Life Sciences, Milford, MA), Virotrol I controls (Bio-Rad Laboratories, France), seroconversion panels and samples for crossreactivity or interference test were also used for evaluation.

Results. The sensitivity and specificity of AFIAS anti-HCV assay were 99.8% (95% CI: 97.1-99.6%) and 99.1% (95% CI: 98.3-99.6%), respectively. The kappa value for the agreement between two assays’ results was 99.0% (k=0.975, 95% CI: 0.962-0.987). AFIAS anti-HCV assay detected all samples with genotype 1, 1a, 1b, 2a, 2b, 4 and 6. Evaluation with 10 seroconversion panels demonstrated the adequate sensitivity. There was no interference or cross-reactivity with other infection.
Tuesday, August 1, 9:30 am – 5:00 pm

Infectious Disease

B-100

Comparison of real-time PCR tests with the routine diagnosis technique to detect enteropathogenic bacteria

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1Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain
2Hospital Clínico Universitario Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, LIS Aragón, Departamento de Microbiología, Zaragoza, Spain
3Hospital Clinico Universitario Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, LIS Aragón, Departamento de Microbiología, Zaragoza, Spain
4CerTest Biotech S.L., Zaragoza, Spain

Background: Infectious gastroenteritis is the most common childhood illnesses worldwide and it is caused by different species of bacteria, viruses and parasites, being Campylobacter, Salmonella, Shigella/EIEC and Yersinia four of the main enteropathogens.

Objective: Compare real-time PCR tests with the routine diagnosis technique to detect enteropathogenic bacteria.

Material/methods: We performed a retrospective study where we tested 400 stool samples from 356 subjects (50% children under the age of 14 years and 50% adults) with gastrointestinal symptoms, from October to December 2015. Total genomic DNA was isolated from stool samples with the “VIASURE RNA-DNA Extraction Kit” (CerTest BioTec S.L). Nucleic acids were amplified on thermocycler AriMix (Agilent Technologies) using the multiplex assay “VIASURE Salmonella, Campylobacter, Shigella/EIEC or Yersinia Real Time PCR Detection Kit” (CerTest Biotec S.L) in comparison to “RIDA®GENE Bacterial Stool Panel” (R-biopharm) and “RIDA®GENE EHEC/EPEC” (R-biopharm). All samples were also amplified using the four monoplex assays: “VIASURE Salmonella, Campylobacter, Shigella or Yersinia Real Time PCR Detection Kit”. Conventional PCR was used to identify the different species of Salmonella and Campylobacter.

Relative to the routine diagnosis method, all samples were cultivated in the routine culture medium. Also MALDI-TOF mass spectrometry was used to identify enteropathogens, and agglutination were used to identify Shigella/EIEC and Yersinia enterocolitica.

Results: 65/400 (16.25%) were true positive for Campylobacter, 42/65 was detected by routine diagnosis, 53/65 by Real Time PCR R-biopharm assay and 65/65 by multiplex Real Time PCR VIAURE assay. Sequencing showed 54.93% Cjejuni, 9.86% C.concisus and 8.45% C.coli.

23/400 (5.75%) were true positive for Salmonella, all of them were detected by routine diagnosis, 21/23 by Real Time PCR R-biopharm assay and 22/23 by Real Time PCR VIAURE assay. Sequencing shows 47.83%S.Typhimurium, 26.09%S. Mbandaka, 4.35% S.Braenderup and 4.35% S. Paratyphi A.

2/400 (0.5%) were true positive for Shigella/EIEC, all of them were detected by routine diagnosis, by Real Time PCR R-biopharm and Real Time PCR VIAURE assay.

4/400 (1%) were true positive for Yersinia enterocolitica O:3, all of them were detected by routine diagnosis, by Real Time PCR R-biopharm and Real Time PCR VIAURE assay.

There is a total concordance between multiplex and monoplex VIAURE PCR assays.

Conclusions: Multiplex assay allows the detection of the three enteropathogens in only one reaction, which reduces the cost and is less consuming. For Salmonella, Shigella/EIEC and Yersinia, “VIASURE Multiplex Real Time PCR Detection Kits” are highly sensitive and specific, being comparable in sensitivity and specificity to culture method and RIDA®GENE Kits. In the diagnosis of Campylobacter, “VIASURE Multiplex Real Time PCR Detection Kits” are the most sensitive one. Molecular techniques have the additional advantage that detects unusual variants of Campylobacter species in comparison to culture method.
Performance Evaluation of the ADVIA Centaur Hbc Total* and Hbc IgM* Assays in the Pediatric Population

B. Plouffe1, D. Desai1, R. Levine1, M. Hilf2, O. De Armas2, Y. De Fuente2, M. De Medina2, E. R. Schiff1. 1Siemens Healthcare Diagnostics, Tarrytown, NY, 2Center for Liver Diseases University of Miami Miller School of Medicine, Miami, FL

Introduction: Hepatitis immunoassays are clinically useful for managing cases of viral hepatitis infection in the pediatric population. This study assessed the performance of ADVIA Centaur® Hepatitis B core Total (HBcT®) and Hbc IgM* assays from Siemens Healthcare Diagnostics Inc., in a pediatric population consisting of children and adolescents.

Objectives: To assess the performance of the ADVIA Centaur HbcT and Hbc IgM assays in a pediatric population presenting with signs and symptoms of hepatitis or at risk for hepatitis B virus infection.

Methods: Concordance of the ADVIA Centaur HbcT and Hbc IgM assays was assessed to comparator assays commercially available in the U.S. and approved for use in children and adolescents. Serum samples from suspected or high-risk pediatric patients were tested in singleton on all assays. Equivocal results were retested, and all test results were interpreted as recommended by the assay manufacturers.

Results: ADVIA Centaur HbcT assay: 62 subjects were included, aged 2 to 21 year-old, with a male/female ratio of 58%/42%, respectively. 12 samples tested positive and 50 negative on the comparator assay. The percent positive and negative agreement were 91.67% (95% CI 61.52-99.79) and 100.00% (95% CI 92.89-100.00), respectively.

ADVIA Centaur Hbc IgM assay: 142 samples were tested, including 107 native samples (58% male and 42% female, age range from 2 to 21 years) and 35 contrived samples. 41 tested positive and 101 negative on the comparator assay. The percent positive and negative agreement were 100.00% (95% CI 91.40-100.00) and 98.02% (95% CI 93.03-99.76), respectively.

Conclusion: The study showed acceptable concordance between the ADVIA Centaur HbcT and Hbc IgM assays and the comparator assays.

*The pediatric claims mentioned herein are not approved in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further detail.

Comparison of two algorithms for the diagnosis of syphilis

H. Hwang, H. Park. Kosin University College of Medicine, Busan, Korea, Republic of

Background: Although the traditional syphilis screening algorithm where nontreponemal screening is followed by confirmatory treponemal testing is recommended in Korea, many laboratories are gradually adopting a reverse syphilis screening algorithm for syphilis testing. We compared the diagnostic performance of the traditional and reverse syphilis screening algorithms in a Korean population.

Methods: A total of 201 patient samples were used. We used HiSens Auto rapid plasma reagin (RPR) test (HBi corp., Korea) as a nontreponemal test, and ADVIA Centaur Syphilis (Siemens, Germany) test and Treponema pallidum antibody (TPab) test (HBi corp., Korea) were used as treponemal tests. ADVIA Centaur Syphilis test was used as a first line test in the reverse syphilis screening algorithm. When the tested samples were positive in TPab and ADVIA Centaur Syphilis test simultaneously, we confirmed the patient samples as an atypical syphilis in this study.

Results: In traditional syphilis screening algorithm, thirty eight patient samples were positive in RPR test, among which 32(84.2%) were positive in TPab and 1 of 32(3.1%) was negative in ADVIA Centaur Syphilis. The rest six (15.8%) were TPab negative. Eleven RPR(-) samples (6.7%) were positive in TPab and 5 among them were positive in ADVIA Centaur Syphilis as well. Thirty six patient samples were positive in the comparator assay tested negative on both the RIBA test and the ADVIA Centaur anti-HCV assay.

Conclusions: The study showed acceptable concordance between the ADVIA Centaur anti-HCV assay and the comparator assay.

*The pediatric claims mentioned herein are not approved in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further detail.

Tuesday, August 1, 9:30 am – 5:00 pm

Clinical Chemistry, Vol. 63, No. 10, Supplement, October 2017 S165
Wednesday, August 2, 9:30 am – 5:00 pm

Infectious Disease

B-107
Performance Evaluation of the ADVIA Centaur HAV Total* and HAV IgM* Assays in the Pediatric Population

B. Plouffe1, D. Desai1, R. Levine1, M. Hill2, O. De Armas3, Y. De La Fuente4, M. De Medina5, E. R. Schiff5. Siemens Healthcare Diagnostics, Tarrytown, NY, 6Center for Liver Diseases University of Miami Miller School of Medicine, Miami, FL

Introduction: Hepatitis immunoassays are clinically useful for managing cases of viral hepatitis infection in the pediatric population. This study assessed the performance of ADVIA Centaur* Hepatitis A Virus (HAV) Total* and HAV IgM* assays from Siemens Healthcare Diagnostics Inc., in a pediatric population consisting of children and adolescents.

Objective: To assess the performance of the ADVIA Centaur HAV Total and HAV IgM assays in a pediatric population presenting with signs and symptoms of hepatitis or at risk for hepatitis A virus infection.

Methods: Concordance of the ADVIA Centaur HAV Total and HAV IgM assays was assessed to comparator assays commercially available in the U.S. and approved for use in children and adolescents. Serum samples from suspected or high-risk pediatric patients were tested in singleton on all assays. Equivocal results were retested, and all test results were interpreted as recommended by the assay manufacturers.

Results: ADVIA Centaur HAV Total assay: 55 subjects were included, aged 2 to 21 years old, with a male/female ratio of 49.09%/50.91%. 11 samples tested positive, 42 tested negative, and 2 tested borderline reactive on the comparator assay. The percent positive and negative agreement were 100.00% (95% CI 71.51-100.00%) and 97.62% (95% CI 87.43-99.94%), respectively. Scoring the two borderline results from the comparative assay as discordant results in the % positive agreement calculation yielded a result of 84.62% (95% CI 54.55-98.08%).

ADVIA Centaur HAV IgM assay: 132 native samples were tested (54.55% male and 45.45% female, age range from 2 to 21 years). 31 samples tested positive, 98 tested negative, and 3 tested borderline reactive on the comparator assay. The percent positive and negative agreement were 96.77% (95% CI 83.30-99.92%) and 98.98% (95% CI 94.45-99.97%), respectively. Scoring the three borderline reactive results from the comparative assay as discordant results in the % positive agreement calculation yielded a result of 90.90% (95% CI 75.67-98.08%).

Conclusion: The study showed acceptable concordance between the ADVIA Centaur HAV Total and HAV IgM assays and the comparator assays.

*The pediatric claims (mentioned herein) are not approved in all countries. Due to regulatory reasons their future availability cannot be guaranteed. Please contact your local Siemens organization for further detail.

B-108
Performance evaluation of the ADVIA Centaur Zika IgM assay*


Background: Siemens Healthineers is developing a fully automated Zika IgM (ZikaM) assay* for the ADVIA Centaur® XP and XPT Immunoassay Systems. The ADVIA Centaur ZikaM assay is an IgM capture two-wash immunoassay using direct chemiluminescent technology. The assay uses recombinant Zika virus NS1 antigen for the qualitative detection of Zika IgM antibodies in serum or plasma.

Methods: The performance of the ADVIA Centaur ZikaM assay was evaluated with serial draw samples obtained from Zika virus PCR-positive individuals plus samples from normal donors (U.S.), pregnant women (U.S.), and symptomatic (Dominican Republic) and asymptomatic (Dominican Republic and Honduras) individuals. Several potentially cross-reactive samples were also evaluated.

Results: ADVIA Centaur ZikaM assay results were reported as reactive for samples with ≥1.00 index and nonreactive for samples with <1.00 index. In 50 Zika PCR-positive serial draw sets (eight draws per individual), all 50 individuals showed reactivity with the ZikaM assay within 2–7 days after the appearance of symptoms, and the majority of these (43/50) were reactive within 14 days post-symptom onset. When the results of all draws from 50 Zika-positive individuals were combined, approximately 85% of the samples were reactive with the ZikaM assay. Evaluation of normal samples from a U.S. population (blood donors and pregnant women) by the ZikaM assay gave a specificity of 94.7% (1418/1497). Specificity in samples collected from Zika-endemic areas was 70.7% (28/41) in individuals with symptoms of Zika and 81.7% (116/142) in asymptomatic individuals. Additionally, cross-reactivity was evaluated using 159 cross-reactive samples, including common flaviviruses (dengue, yellow fever vaccinees, and West Nile).

* Under Development
**B-109**

**Discrimination Between Viral and Bacterial Etiology of Lower Respiratory Tract Infection Using Cell Population Data Generated by Unicel DxH800 Coulter Cellular Analyzer**

T. Cevlik1, R. Turkal1, S. Satilmis2, A. Ilki2, O. Sirikci1, G. Haklar3, 1Biochemistry Laboratory, Marmara University Pendik E&R Hospital, Istanbul, Turkey, 2Dept. of Microbiology, Istanbul, Turkey, 3Biochemistry, School of Medicine, Marmara University, Istanbul, Turkey

**Background:** Discrimination of viral vs. bacterial etiology of lower respiratory tract infection is critical. Although WBC and differentials may provide useful information for this discrimination, the sensitivity and specificity of these parameters are not satisfactory. Likewise, C-reactive protein and procalcitonin are not completely satisfactory. Other tests, such as blood culture for bacteria or molecular/antigen studies for viruses are time-consuming, expensive, and labour intensive. The Unicel DxH 800 Coulter Cellular Analyzer with volume, conductivity, and light scatter (VCS) technology generate cell population data (CPD) encompassing morphologic properties of leukocytes. Our aim was to evaluate the usefulness of CPD for the differential diagnosis of viral and bacterial infection etiology in lower respiratory tract infections.

**Methods:** Complete blood count and CPD data measured in the Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA) were collected retrospectively from 65 patients with a diagnosis of viral infection, acute bacterial infection with (+) sputum culture (n=21), and 106 (viral Ag negative 84 and sputum culture negative 22 cases) controls. Viral infection was diagnosed by *combo card test* (CerTest, Biotec, Spain), which is a coloured chromatographic immunoassay for the simultaneous qualitative detection of *RSV* and *Adenovirus* antigens. For bacterial diagnosis, sputum samples were stained by Gram stain, and were inoculated on 5% sheep blood agar, Chocolate Agar, Haemophilus Agar, MacConkey Agar (BioMerieux, France). After overnight incubation, the samples were evaluated. The CPD data include mean (MN) and standard deviation (SD) of volume of lymphocytes and neutrophils, and lymphocyte conductivity. Lymphocyte (LI) index was calculated. Results: WBC of bacterial culture (+) and viral Ag (+) cases were not different significantly different (7.8, range 6.7-12.3 and 11.5, range 7.5-12.2; p=0.074, respectively). Mean percent neutrophils were significantly higher in bacterial culture (+) cases 75.7 vs 43.9, P<0.001, on the other hand mean percent lymphocytes were higher in viral Ag (+) cases 12.9 vs 40.3, P<0.001. The lymphocyte conductivity (LcS) was significantly higher in the viral infection than the bacterial infection (P=0.004). In ROC analysis, lymphocyte volume SD (LySDV) sensitivity 71% specificity 63.1, LyC sensitivity 71% specificity 59.4 in diagnosing viral infection.

**Conclusion:** We concluded that for the discrimination of bacterial and viral infections, CPD parameters merits further exploration in larger prospective studies.

**B-110**

**Ready-To-Use Real-Time PCR-Based Assay for the Detection of Human Herpesvirus 6**

M. Grameggia, M. Ballarini, L. Turner, M. Incandela, L. Spinelli, D. Rigamonti, A. Moiana. Sentinel CH, Milano, Italy

**Background:** Human Herpesvirus 6 (HHV-6) is a set of two closely related herpes viruses known as HHV-6A and HHV-6B. HHV-6B infects nearly 100% of human beings, typically before the age of three. Like other herpesviruses, HHV-6 establishes lifelong latency and can become reactivated later in life. Reactivation occurs mostly in transplant patients taking immunosuppressant drugs or individuals with immune deficiencies and it can involve brain, lungs, heart, kidney and gastrointestinal tract. Both HHV-6 viruses are highly cell associated and can be detected in plasma briefly during the initial infection or acute reactivation. Detection of HHV-6 DNA in plasma generally means the patient has an active infection. The aim of this work was to evaluate the performance of a new assay designed to detect HHV-6 DNA in human samples.

**Methods:** The assay was developed as a ready-to-use test containing all the required elements for the amplification of both HHV-6 DNA fragment and human beta-globin gene as internal control. The two sets of primers and probes are combined in a lyophilized and ready-to-use mix, co-amplified and detected by a Real-Time PCR instrument. In the present study, several samples obtained from San Raffaele Hospital, previously tested with an “in-house” Real-Time PCR test, were investigated. PCR reactions were performed on nucleic acids extracted from plasma, whole blood and cerebrospinal fluid (CSF).

**Results:** All tested samples were previously diagnosed as positive. The new freeze-dried ready-to-use assay demonstrated robust and accurate target amplification, according to the data obtained at San Raffaele Hospital. This detection limit was found to be specific for HHV-6. The assay did not cross-react with any of the other Human Herpesviruses tested.

**Conclusion:** The described Real-Time PCR assay proved its effectiveness for the detection HHV-6 DNA in samples. The test showed a sensitivity and a specificity of 100%. The high-sensitivity and specificity of this assay, associated with the ready-to-use and room temperature storage, would have a direct impact on the early and correct management of the affected patients.

**Clinical Chemistry, Vol. 63, No. 10, Supplement, October 2017**

**S167**
Molecular characteristics of HBV virus in patients with HIV/HBV combined infection

T. Wang, D. Li, Q. Chen, C. Tao*, S. Feng, C. Rao. West China Hospital of Sichuan University; Chengdu, China

Background: HBV and HIV share common routes of transmission, so HIV/HBV coinfection is common. We analyzed patients with HIV/HBV coinfection and HBV mono-infection cases, and compared the HBV genotype distribution, RT and BCP/PC mutation rate.

Materials and Methods: 54 patients with HIV antibody and HBV DNA>10 IU/ml were defined as the research group, while 56 cases without HIV antibody and with HBV DNA>10 IU/ml as the control group. HBV DNA was extracted from serum or plasma. Then, nested PCR was performed for the RT and BCP/PC region. After electrophoresis, PCR products were sequenced. The sequencing results were analyzed.

Results: Genotype B was the dominant genotype in HIV/HBV coinfection patients, accounting for 81.48%, while genotype C accounted for 14.82%. The other two cases were C/D recombinant. The total drug resistance rate was 3.70%. A1762T/G1764A and G1896A mutations were the highest mutations in BCP/PC region. A1762T/G1764A mutation rate was 35.19%, and G1896A mutation rate was 59.30%. Compared with HBV mono-infection group, there was no significant difference in HBV genotype distribution, drug resistance rate, HBV gene system evolutionary tree and PC mutation rate. And in HIV/HBV coinfection group A1762T/G1764A mutations rate was lower. A1762T/G1764A and G1896A mutation rate of HBVAg negative patients was higher than the HBVAg positive patients for both HIV/HBV coinfected group or mono-HBV infection group.

Conclusion: This study was the first time that explored the molecular characteristics of HIV/HBV coinfected patients and the similarities and differences of the HIV/HBV coinfected and HBV mono-infected persons in molecular biology. Research results showed that the genotypes of both groups were mainly genotype B and C, low resistance rates, similar Phylactic evolution, high BCP/PC mutation rate. Yet, HBV mono-infected persons has higher A1762T and G1764A mutation.

| HBV genotype comparison of HIV/HBV coinfection group and HBV mono-infection group |
|---------------------------------|-------------|
| HIV/HBV coinfection (n=54)      | HBV mono-infection (n=56) |
| Genotype B                      | Genotype C   |
| 44(81.48%)                      | 10(18.62%)   |
| Genotype C                      | Genotype D   |
| 10(18.62%)                      | 2(3.70%)     |
| Recombinant C/D                 |              |
| 0                               | 0            |

Mcr1 gene: first detected case in Ceará, Northeastern Brazil

M. C. Gurrel Castelo1, S. P. Bandeira2, L. L. A. Camaleante2, E. J. U. Kusano1, A. C. C. Pignataro2, F. M. P. Ventura2, R. R. Araujo1, T. A. C. Palhano1, G. A. Campana1, E. S. Girão1, (1Universidade Federal do Ceará; DASA, Fortaleza, Brazil; (2Universidade Federal do Ceará; DASA, Fortaleza, Brazil; (3Universidade de São Paulo, Brazil; (4Universidade Federal do Ceará; Fortaleza, Brazil

Background: Resistance to polymyxins is already known in Gram negative bacteria due to mutation in chromosomal sites. In 2015, Liu et al. described mcr1 gene as responsible for plasmid-mediated resistance to polymyxins in Escherichia coli strains. Since then, some cases have been described in several countries worldwide. However, few cases have been reported in Brazil and no reports of this gene have been described in strains from Ceará, Brazil.

Methods: This work aims to describe the first case of detection of mcr1 gene in Escherichia coli from Ceará, Brazil.

Results: A 86-year-old female patient was admitted to the hospital with complaints of general decline and drowsiness for 3 days. She had reported sudden respiratory distress. She was transferred to ICU and it was prescribed Piperacillin-Tazobactam EV. Urine culture and hemoculture were requested. Urine sample was seeded in CPS ID3 agar (BioMerieux™). After 24 hours at 35°C, a pink colony was detected (figure 1a). Analysis by Vitek 2 (BioMerieux™) resulted in Escherichia coli identification with resistance pattern to ampicillin, ciprofloxacin and colistin. The MIC found for colistin was 1ug/mL. E-test of polymyxin was performed, which confirmed in vitro resistance pattern (figure 1b). The isolate was subjected to real-time PCR and the presence of mcr1 gene was detected.

Conclusion: This is the first described case of detection of mcr1 gene from Ceará, Brazil. Since it is a plasmid-mediated resistance mechanism, early detection of this gene is desirable in order to prevent horizontal dissemination of resistance to other bacterial species, which may limit available therapeutic arsenal for infections by multidrug resistant microorganisms.
### Study of the concordance of serological tests for anti-Leishmania antibodies detection

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Background: Visceral Leishmaniasis (VL) is a potentially fatal endemic zoonosis, if not promptly diagnosed and treated. In the presence of suggestive clinical and laboratory data, a reactive immune test reinforces the diagnosis of VL. Indirect immunofluorescence (IF) and ELISA are the serological tests most frequently used for the serological diagnosis of VL. Unfortunately, the knowledge about the agreement of available commercial assays for anti-Leishmania antibodies detection is limited. Objective: To evaluate the agreement between the commercial kits: Novatec (ELISA), EUROMMUN (IF) and R-Biopharm (ELISA) for anti-Leishmania antibodies detection. Methods: A total of 84 samples obtained in Institute Hermes Pardini routine were selected from results of R-biopharm kit (comparative method), being 40 negative and 44 positive samples. Fifty-five samples were also tested in the IT-LEISH kit (Gold standard). Results: The Kappa index was 0.352 (R-Biopharm and EUROMMUN, 95% CI= 0.206 to 0.498), indicating regular agreement; 0.476 (R-biopharm and Novatec, 95% CI= 0.327 to 0.626), indicating moderate agreement and 0.607 (Novatec and EUROMMUN, 95% CI= 0.426 to 0.788), indicating moderate agreement. The main causes of disagreement were: positives results by R-Biopharm Kit and negatives by Novatec Kit (n=20), positives results by R-Biopharm kit and negatives by EUROMMUN kit (n=27). The Novatec and EUROMMUN kits presented the higher degree of agreement. The results of each kit compared to gold standard method (IT-LEISH) are shown in Table 1. The sensitivity and specificity of each kit are described as follows, respectively: R-Biopharm kit = 94% and 32%; Novatec kit = 94% and 81%; EUrommun kit = 67% and 89%. Conclusions: The absence of a good level of agreement between the kits evaluated suggests a lack of standardization among the commercial immunological tests available for the serological diagnosis of VL. Thus, is necessary to develop a standard diagnostic method for VL diagnosis, such as serological tests used by reference centers.

#### Table 1: Agreement of kits compared to IT-LEISH.

<table>
<thead>
<tr>
<th>Kit</th>
<th>IT-LEISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-biopharm</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
</tr>
<tr>
<td>Novatec</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
</tr>
<tr>
<td>Intermediate</td>
<td>3</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>EUrommun</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>33</td>
</tr>
<tr>
<td>Intermediate</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>3</td>
</tr>
</tbody>
</table>

### Evaluation of Interference between Biotin and the Streptavidin-Biotin-Based VITROS Hepatitis A-Specific Total and IgM Antibody Immunoassays

J. C. Jara Aguirre, J. D. Yao, N. A. Baumann, B. M. Katzman, T. J. Stier, E. S. Theel. Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

Background: Interference of biotin with certain streptavidin-biotin-based immunoassays has been described for plasma and serum specimens from patients taking oral biotin supplements for certain inherited metabolic diseases, demyelinating diseases, or to enhance hair and nail growth. Such interference may cause false-positive (over-estimation) or false-negative (under-estimation) results in competitive and non-competitive immunoassays, respectively. We conducted a study to investigate the effect of biotin interference in 2 streptavidin-biotin-based, serologic assays, VITROS Anti-HAV Total (HAVT) and VITROS Anti-HAV IgM (HAVM) (Ortho-Clinical Diagnostics, Inc.), used for detection of hepatitis A-specific total antibodies (combined IgG and IgM) and IgM antibodies, respectively.

Methods: HAVT and HAVM are competitive and non-competitive immunoassays, respectively, performed with the fully automated VITROS 3600 ImmunoDiagnostic System (Vitros 3600; Ortho-Clinical Diagnostics, Inc.). Aliquots of serum were prepared from pooled clinical specimens with 4 different signal/cut-off (S/CO) ratio for HAVT: 1.74 (negative), 1.25 (positive), 0.550 (reactive), and 0.030 (reactive). Aliquots of serum were prepared similarly with 3 different S/CO ratio for HAVM: 0.010 (negative), 1.67 (reactive), and 6.86 (reactive). These aliquots were spiked with a 0.9% NaCl solution containing 10,000 ng/mL biotin (Sigma-Aldrich) to achieve final biotin concentrations of 0, 10, 50, 100, 500 and 1,000 ng/mL. Each concentration of biotin in serum was tested in duplicate for HAVT and HAVM on VITROS 3600 per manufacturer’s instructions for use. A difference of >14% between observed and expected S/CO ratios was considered significant change, based on manufacturer’s precision data for these two assays.
Wednesday, August 2, 9:30 am – 5:00 pm

**Infectious Disease**

Results: Observed S/CO ratios of HAVT and HAVM at various ion concentrations are shown below:

<table>
<thead>
<tr>
<th>Assay</th>
<th>Expected qualitative result</th>
<th>Mean S/CO ratio observed in serum aliquots with the following ion concentrations in ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAVT</td>
<td>Negative</td>
<td>1.74 ± 1.75 ± 1.60 ± 0.980 ± NT ± NT</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1.25 ± 1.17 ± 0.990 ± 0.660 ± 0.290 ± 0.120</td>
</tr>
<tr>
<td></td>
<td>Reactive</td>
<td>0.550 ± 0.540 ± 0.470 ± 0.340 ± 0.050 ± 0.035</td>
</tr>
<tr>
<td></td>
<td>Reactive</td>
<td>0.050 ± 0.030 ± 0.025 ± 0.020 ± NT ± NT</td>
</tr>
<tr>
<td>HAVM</td>
<td>Negative</td>
<td>0.010 ± 0.010 ± 0.010 ± 0.010 ± 0.010 ± 0.010</td>
</tr>
<tr>
<td></td>
<td>Reactive</td>
<td>1.67 ± 1.67 ± 1.63 ± 1.60 ± 1.64 ± 1.62</td>
</tr>
<tr>
<td></td>
<td>Reactive</td>
<td>0.66 ± 0.65 ± 0.65 ± 0.67 ± 0.65 ± 0.69</td>
</tr>
</tbody>
</table>

*Change in qualitative interpretive result; NT, not tested.

Conclusion: HAVT was more susceptible than HAVM to ion-tolerance, leading to a clinically significant change in qualitative interpretive results of original borderline negative specimens. Clinical laboratories should recognize and evaluate potential ion-tolerance of ion streptavidin-biotin-based immunoassays.

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**B-120**

Evaluation of Serologic Laboratory Test in Brazilian Patients Infected by Zika virus and exposed to Dengue virus and Chikungunya virus.

D. A. G. Zauli, N. Nunes, W. Pedrosa, A. C. S. Ferreira, E. Mateo, Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil, Hermes Pardini Institute, Vespasiano, Brazil

Background: Zika virus (ZIKV) is virus whose newest pandemic is alarming, although, with the paucity of literature the exact details of the disease are not clear. The diagnosis of ZIKV infections can be performed on clinical-epidemiological and laboratory bases. Overall, your laboratory diagnosis relies on the same usual strategies used for other arboviruses, with viral genome detection by RT-PCR tests on acute-phase samples and serology (ELISA and immunofluorescence). Usually, the choice of the laboratory approach used will depend on the goal of the analysis, laboratory infrastructure, technical expertise and sampling availability. The diagnosis of ZIKV infection performed by serological tests can detect specific IgM or IgG antibodies against ZIKV after 5 to 6 days of the onset of symptoms, with increased titers within 2 weeks. Objective: To evaluate the performance of commercial Euroimmun ZIKV ELISA test (Euroimmun, Lübeck, Germany) using panels from Brazilian patients exposed to Dengue (DENV) and Chikungunya (CHIKV) infection.

Method: Samples were obtained in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) routinely, with a high potential of causing cross-reactions in serological flavivirus assays. The selection of samples was based on six panels for IgM analysis (n=105) and four panels for IgG analysis (n=65). For IgM assay the panels were as follows: Panel I: 20 DENV IgM-positive samples; Panel II: 15 DENV IgM and IgG-negative samples; Panel III: 15 CHIKV IgM-positive samples; Panel IV: 15 CHIKV IgM and IgG-negative samples; Panel V: 20 Epstein Barr virus IgM-positive samples; Panel VI: 20 Rheumatoid Factor (RF) IgM-positive samples (Concentration: 153.5 to 1530.9 IU/mL). For IgG assay the panels were as follows: Panel I: 10 DENV IgG-positive; Panel II: 15 DENV IgM and IgG-negative samples; Panel III: 15 CHIKV IgM-positive samples; Panel IV: 15 CHIKV IgM and IgG-negative samples. All assays were performed according to the instructions of the manufacturer. Results: The ZIKV IgM ELISA was negative in all simultaneously DENV and CHIK negative samples, CHIK positive, EBV positive and RF positive samples. Of DENV positive samples 10% were ZIKV IgM positive (2/20). For IgG ELISA, 20% of DENV positive samples were ZIKV IgG positive (4/20). All simultaneously DENV IgM and IgG negative samples were ZIKV IgG negative. Of CHIKV IgG positive samples 60% were ZIKV IgG positive (9/15). In addition, 33% of simultaneously CHIKV IgM and IgG negative samples were ZIKV IgG negative (5/15). Conclusion: Positive samples for the dengue virus may also be positive for ZIKV. No cross-reactivity of IgM ZIKV with Chikungunya virus has been observed. The IgG positive samples for dengue and Chikungunya viruses may be positive for ZIKV. Despite this it is not established if this situation corresponds to a cross reaction of the test or simply histories of coinfection in different periods in the same individual in endemic areas. Its positivity should be evaluated in the context of the other conditions, such as Epstein Barr virus infections, and in malaria infection. Further studies will be necessary to determine the accuracy of this test and other current assays in a larger set of well-defined samples.

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**B-121**

Development and evaluation of a rapid POC multiplex test for the detection of Zika, Dengue and Chikungunya antibodies in patient’s blood samples.


The Chembio Diagnostics proprietary rapid POC DPP® platform is well suited for high sensitivity and multiplex detection of antigens or antibodies in a variety of body fluids. Here, the DPP platform has been expanded to include two test strips in a single cassette, sharing a single sample of fingerstick blood to detect IgM antibodies and IgG antibodies against three possible infectious diseases: Zika, Chikungunya and Dengue. Results are obtained in a few seconds using a small, portable reflectance reader. Differentiating IgM and IgG provides information on the disease phase. This multiplex assay is needed worldwide as the three viruses are carried by the same mosquito vectors and present similar symptoms at the time of infection. Sensitivity of the DPP Zika/Chikungunya/Dengue IgM/IgG System (“DPP Z/C/D”) was evaluated against ELISA for IgM and IgG against each of DENV, CHIKV and ZIKV. DENV of 57 plasma samples, positive for Dengue IgM and/or IgG antibodies, 16 tested positive for DPP Z/C/D, and 15 tested positive by ELISA. Of 46 samples, positive for IgG on DPP Z/C/D, 45 specimens tested positive by ELISA.ZIKV: For IgM, of 24 ZIKV PCR+ serum samples, 7 were positive on DPP Z/C/D, 13 by ELISA, 4/8 plasma samples were positive for ZIKV IgM on DPP Z/C/D, and only 2 specimens by ELISA. For IgG, 24/24 serum samples tested positive on DPP Z/C/D, 7 were reactive by ELISA. 6/8 plasma samples were reactive for Zik IgM on DPP Z/C/D; 7/8 were reactive by ELISA.CHKV: Of 54 samples, positive for IgM on the DPP Z/C/D, 51 tested positive for IgM by ELISA. Of 59 samples, positive for IgG by ELISA, DPP Z/C/D found 54 positives. Specificity of the DPP Z/C/D assay was evaluated with 50 whole blood/EDTA specimens and yielded between 94 and 100% specificity for the three analytes. These results suggest that the Chembio Dengue/ Zika/Chikungunya IgM/IgG System provides an effective, rapid POC solution with performance equivalent to ELISA, in the field and at the patient’s side.

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**B-122**

An Evaluation of Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay


Background: This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products HIV Combo Assay (VITROS HIV Combo Assay®) on the VITROS® 3600 Immunodiagnostic System. The assay is capable of simultaneous detection of HIV antibodies (Ab) and HIV p24 antigen (Ag) to help enable earlier diagnosis of HIV infection.

Methods: Antibody detection in the VITROS HIV Combo Assay® is achieved using recombinant transmembrane antigens specific to HIV-1 (group M and O) and to HIV-2. The p24 antigen detection is accomplished using monoclonal antibodies (MAB). The antigens and p24 MAB are coated on the wells in the first stage of the reaction, and HIV Ag/Ab from the sample is captured. After washing, HRP conjugated antigens and p24 MAB are added, 2 washes and a final wash, bound HRP conjugates are detected using the VITROS signal reagent. Specificity was assessed using 6435 samples from low risk populations. Supplemental testing to determine HIV status was conducted on all reactive samples. Sensitivity was evaluated using 1764 antibody positive samples (1535 HIV-1, including 413 from various Group M and O subtypes and 229 HIV-2). In addition, 52 samples with various HIV-1 group M antigen genotypes were tested. Seroconversion sensitivity was assessed by testing 32 commercially available panels on both the VITROS HIV Combo assay and a commercially available Ag/Ab assay. Assay reproducibility was assessed at three sites using three reagent lots with a 14 member panel. Antigen sensitivity was determined by testing serial dilutions of the NIHSC and the AFSAPS HIV-1 p24 Ag standards across two reagent lots.

Results: The specificity of the VITROS HIV Combo Assay® for the low risk population was 99.59% (6365/6391) [95% exact CI (99.40-99.73%)]. Samples confirmed as HIV positive by supplemental testing were removed from the analysis. The sensitivity for HIV-1 and HIV-2 antibody positive samples was 99.94% (1763/1764) [exact 95% CI (99.68-100.00%)]. All 413 HIV-1 group M and O antibody positive subtypes and 50 of 52 HIV-1 group M antigen genotypes were reactive with the VITROS HIV Combo assay®. For seroconversion panels the VITROS HIV Combo

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**Infectious Disease**

**B-123**

AccuSpan Zika Linearity Panel Spurs the Dynamic Range of Assays and Allows Evaluation of Analytical Sensitivity

H. Lee, V. MacKeen, J. Wu, R. Vemula, C. Huang, B. Aneksela, SeraCare Life Sciences, Milford, MA, 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 throughout countries of the Americas and more recently Pacific island nations, as well as its link to neurological birth defects. In response to this outbreak, several PCR-based Zika assays have been developed and approved under FDA’s Emergency Use Authorization. Clinical laboratories as well as test developers need to evaluate assays across the entire reportable range for sensitivity and linearity. SeraCare has developed the Accuspan Zika Linearity Panel to meet this need. The panel uses recombinant Sindbis virus technology; therefore, it is non-infectious but is a whole virus antigen for each recombinant virus construct (Envelope region, NS2/NS3 region, NS4 region) were designed and used for quantitation of each viral stock. Based on digital PCR quantitation, the viral stocks were mixed together in equimolar ratios such that the combined bulk contained sequences of the entire Zika genome. The combined bulk was then serially diluted in defibrinated human plasma to titers of 1.0E+06, 1.0E+05, 1.0E+04, 1.0E+03, 1.0E+02, and 1.0E+01 copies/mL. Titers of each member were verified by digital PCR testing. A negative member (containing the plasma diluent) is also included in the panel.

**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 a recombinant virus using Sindbis vector system. The recombinant 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. Digital PCR assays, specific for each recombinant virus construct (Envelope region, NS2/NS3 region, NS4 region and NS5 region) were designed and used for quantitation of each viral stock. Based on digital PCR quantitation, the viral stocks were mixed together in equimolar ratios such that the combined bulk contained sequences of the entire Zika genome. The combined bulk was then serially diluted in defibrinated human plasma to titers of 1.0E+06, 1.0E+05, 1.0E+04, 1.0E+03, 1.0E+02, and 1.0E+01 copies/mL. Titers of each member were verified by digital PCR testing. A negative member (containing the plasma diluent) is also included in the panel.

**Results:** Panel member testing was performed on various assays, both approved for Emergency use and in development including the Roche LightMix® Zika rRT-PCR Test, Hologic Aptima® Zika Virus Assay, and Beckman DXN® Zika Virus Assay - EUA (pending FDA authorization). Testing results indicate these assays all have lower limits of detection of 100 copies/mL or lower. More extensive testing was performed on Roche Zika Assay for the 1.0E+03 and 1.0E+02 copies/mL Panel members. Members were tested in five replicates on three days; all replicates were positively detected. The 1.0E+03 copies/mL member was detected with average cycle threshold value of 30.76 ± 0.099 and the 1.0E+02 copies/mL member was detected with average cycle threshold value of 33.83 ± 0.39.

**Conclusions:** SeraCare has developed a stable, well-characterized linearity panel for Zika virus. This panel will enable laboratories to validate tests and ensure test performance to improve preparedness. The Accuspan Zika Linearity panel demonstrates the utility of recombinant virus technology to produce non-infectious reference materials for dangerous viruses that are difficult to source or propagate.

**B-125**

A Rapid Syphilis Test for Qualitative Measurement of Treponemal Antibody

C. Xu, Q. Shi, ZBS Corporation, Toronto, ON, Canada

**Background:** Syphilis is a sexually transmitted disease caused by bacteria called Treponema pallidum (TP). Most people with syphilis tend to be unaware of their infection and they can transmit the infection to their sexual contact or in the case of pregnant woman, to her unborn baby. If left untreated, syphilis can cause serious consequence such as stillbirth. According to WHO, syphilis kills more than one million babies a year worldwide, and 12 million new cases of syphilis occur every year. In addition, as a cause of genital ulcer disease, syphilis has been associated with an increased risk of HIV infection. Early diagnosis is important because timely treatment of syphilis significantly prevents its serious consequence and the spread of the disease. An easy-to-use rapid test for the detection of Treponemal antibody can facilitate an early diagnosis of a syphilis. The objective of this study is to evaluate the performance of a new syphilis rapid test. **Principle:** The ADEXUS-Dx Syphilis Rapid Test ("Syphilis Test") is a solid phase immunochromatographic assay. The Syphilis Test uses a sandwich format to detect the presence of Treponemal antibody in blood, plasma, and serum samples. The appearance of a purplish-red band in the test window indicates that the sample contains Treponemal antibody. The Syphilis Test has a unique feature of finger-stick, capillary whole blood sampling needing only 35 µl blood. No additional buffer is needed. The result is ready in 15 minutes.

**Performance:** 100 known Treponemal antibody positive samples were tested by the ADEXUS-Dx Syphilis Rapid Test and 99 tested positive. The calculated clinical sensitivity is 99%. Within these 100 positive patient samples, 51 are serum, 26 are EDTA plasma, 11 are heparin plasma, and 12 are citrate plasma. The expected results were obtained for both serum and plasma samples, indicating the test was unaffected by anticoagulants. 100 known Treponemal antibody negative serum samples were tested by the ADEXUS-Dx Syphilis Rapid Test and 94 tested negative. The calculated clinical specificity is 94%. Sera containing human anti-mouse antibodies (HAMA) up to 327 ng/mL tested negative suggesting minimal interference by HAMA in a normal population based on the reference range for HAMA (0-188 ng/mL). The Syphilis test was compared with a FDA CLIA waived test, Syphilis Health Check, which is also a lateral flow test. Ten known Treponemal antibody positive samples were tested by both methods and 100% agreement (all positive) was achieved although ADEXUS-Dxs Syphilis Rapid Test showed relatively stronger signal. Testing of 5 normal fresh capillary blood from finger pricks showed negative results. **Conclusion:** The Syphilis Test is a one-step rapid test with clinical sensitivity and specificity 99% and 94% respectively. No HAMA interference was observed. The fact that the new test is suitable to test capillary blood makes this test a potential point of care and over the counter Syphilis Rapid Test.

**B-126**

Performance Evaluation of the HIV Ag/Ab Combo (CHIV) Assay on the Siemens ADVIA Centaur XP System

A. Macumber, K. W. Simkowski, Beaumont Health System, Royal Oak, MI

**BACKGROUND:** A limited number of 4th Generation (4G) HIV tests is currently available to detect both HIV-1 and HIV-2 antibodies and the p24 antigen (HIV-1). Our hospital system currently uses the Abbott ARCHITECT® 4G immunoassay (IA) with reagent by BioRad Multispot HIV-1/HIV-2 Rapid Test kit for routine testing. When the Multispot does not confirm a repeatedly reactive ARCHITECT result, serum is referred for further HIV-1 RNA qualitative testing.

**OBJECTIVE:** The Siemens ADVIA Centaur HIV-1/2 Ag/Ab Combo (CHIV) was recently approved by the FDA. The both the ARCHITECT and ADVIA Centaur report a signal-to-cutoff ratio for combined HIV-1/2 antibodies and p24 antigen (HIV-1). The Abbott ARCHITECT is a manual-loading, stand-alone instrument while the ADVIA Centaur is attached to the Siemens Lab Cell automated line. Therefore, our interest was to evaluate the ADVIA Centaur method to replace the current ARCHITECT method for routine testing.

**STUDY DESIGN:** Remnant sera from 200 patients (100 HIV-1/2 Ag/Ab reactive and 100 nonreactive) originally screened by the ARCHITECT HIV Ag/Ab Combo assay were obtained, re-run on the ARCHITECT and then run on the ADVIA Centaur by the HIV Ag/Ab Combo (CHIV) assay. All repeatedly reactive samples were clarified by performing the BioRad Multispot HIV-1/HIV-2 Rapid Test. All samples initially repeatedly reactive by the ARCHITECT method and nonreactive by the Multispot were sent to a reference lab for HIV-1 Qualitative RNA testing performed by transcription mediated amplification for further clarification of HIV status.

**METHODS:** ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay is an in vitro diagnostic immunoassay for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV type 1 (including group “O”) and type 2 in serum. ARCHITECT HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay (CMIA) for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV type 1 (group M and group O) and/or type 2. **RESULTS:** Inter-assay precision for all positive and negative controls were acceptable. Out of the 100 HIV-1/2 Ag/Ab reactive specimens, 76 were reactive for HIV-1 antibodies, 1 sample was HIV-2 antibody reactive and 1 sample was reactive for HIV-1 antigen.
by both the ARCHITECT and ADVIA Centaur. There were 22 (11%) discrepant HIV Ag/Ab samples between the two methods (as determined by supplemental testing on the BioRad Multipost and HIV-1 Qualitative RNA testing). All 22 samples were deemed nonreactive by supplemental testing and nonreactive by the ADVIA Centaur XP method but repeatedly reactive by the ARCHITECT method. All 100 HIV-1/2 Ag/Ab nonreactive specimens were nonreactive on the ARCHITECT and ADVIA Centaur for 100% negative agreement. No supplemental testing was performed on these samples.

CONCLUSIONS: Based on supplemental testing, it appears the ADVIA Centaur CHIV method demonstrates fewer false positive results as compared to the ARCHITECT 4G HIV testing. Further review of patient histories is warranted to determine if patients were re-tested at 1 to 6 months based on recommendations for repeatedly reactive HIV screening results and nonreactive supplemental testing. The Siemens ADVIA Centaur XP CHIV method would be an acceptable replacement for HIV-1/2 Ag/Ab routine testing.

B-128
A New One-Step Direct-Sampling Hepatitis B Surface Antigen Rapid Test
M. Tsang, Q. Shi. ZBx Corporation, Toronto, ON, Canada

Background: Hepatitis B surface antigen (HBsAg) is a group of protein complexes found in serum of patients with hepatitis B virus (HBV) infection and indicates a current hepatitis B infection. HBsAg detection by immunoassay is used in blood screening, to establish a diagnosis of hepatitis B infection in the clinical setting (in combination with other disease markers) and to monitor antiviral treatment. HBsAg usually appears 4 weeks after viral exposure but can be detected any time after the first week. An individual positive for HBsAg is considered to be infected. Persistence of HBsAg is used to differentiate acute from chronic infection. Presence of the antigen longer than 6 months after initial exposure indicates chronic infection. However, the level of antigen does not appear to correlate with disease severity. A rapid one-step test format that accepts a small volume of whole blood will facilitate point-of-care as well as self-testing for HBV infection. The objective of this study is to demonstrate the clinical utility of a new one-step HBsAg antigen test. Principle: The ADEXUS-Dx HBsAg Test was developed using a direct sampling immunoassay technology for whole blood, plasma or serum. Monoclonal antibodies to HBsAg were employed for the qualitative detection of HBsAg. A small sample volume (35-40µL) is required to run the test and no extra buffer is needed. Capillary blood from a finger tip can be directly applied to the test without any transfer device. When the sample is sufficient to fill the Receiving Channel, the sample flows into a dry porous test strip composed of a membrane array with gold conjugated HBsAg antibodies. The appearance of a visible purplish-red band at the test region indicates the sample contains a detectable level of HBsAg. Performance: The new one-step rapid HBsAg test requires less than 40µL of sample and was completed in 15 minutes without additional steps. The test did not have any high dose “hook” effect up to 20 µg/ml of recombinant HBsAg. Samples containing human anti-mouse antibodies up to 327ng/mL measured by Abzyme did not produce false positive results. The test analytical sensitivity is 3.0 IU/mL, based on the Third WHO International Standard for HBsAg (HBV genotype B4, HBsAg subtypes ayw1/adw2). Clinical sample testing showed that the test recognized HBsAg subtypes adw, ayw, adr, and ayr. A total of 188 clinical samples (78 positives and 110 negatives measured by Abbott EIA) were tested. The overall relative sensitivity and specificity for the detection of HBsAg were 96% and 94% respectively. Fresh normal capillary blood test showed negative results with good plasma separation. Conclusion: The ADEXUS-Dx HBsAg Test is a true one-step rapid test suitable for capillary blood testing. It demonstrated good sensitivity and specificity. It is suitable for use in the detection of HBV infection at the point-of-care settings and for self-testing.

B-129
Identifying Significant Association between Host Factors and Improvement of QF TB-Gold Testing with Decreased Incubation Periods
D. Elioum, M. R. Al-Turkmani, L. V. Rao. University of Massachusetts Medical School, Department of Pathology, Worcester, MA

Background: Quantiferon-TB Gold In-Tube test (QF) can be used as an alternative to tuberculin skin testing (TST) for the targeted testing of latent tuberculosis and detection in adults previously exposed to BCG vaccine. Due to many shortcomings with TST, QF usage is increasing. QF has improved specificity, less reader bias and increased cost effectiveness. Shortcomings of the QF include variable sensitivity and high rates of indeterminate results in certain groups. Indeterminate results can be due to manufacturing defects, preanalytical error- in collection, delayed incubation or inadequate processing of the sample, or confounding patient factors, such as immunosuppression. In this study we aimed to evaluate the effect of shorter incubation periods on the rate of indeterminate results as well as to assess the correlation of indeterminate results with multiple clinical variables before and after shortened incubation periods.

Methods: In January of 2016, our institution implemented a one hour incubation period for Quantiferon-TB Gold samples, replacing the previous 6 hour incubation period maximum. In this study, we retrospectively identified 112 patients who received QF testing in the 5 months prior to implementation and 141 patients who received QF testing in the 12 months after implementation of 1hr incubation. Seven clinical risk factors associated with indeterminate results were evaluated.

Results: The rate of indeterminate results at our institution dropped from 11.8% to 5.3% after implementation of 1-hour incubation periods. The distribution and clinical characteristics of patients are summarized in Table 1.

Conclusion: While shortening incubation period from 16 hours to 1 hour resulted in a significant decrease in the rate of indeterminate test results in all risk factor categories, significant associations were found between hypoalbuminemia and cirrhosis and the decrease in indeterminate rates with shortened incubation.

Table 1:

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>16-Hour incubation period</th>
<th>1-Hour incubation period</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoalbuminemia</td>
<td>45.7%</td>
<td>65.2%</td>
<td>0.040</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>21.7%</td>
<td>40.9%</td>
<td>0.034</td>
</tr>
<tr>
<td>Malignancy</td>
<td>13.0%</td>
<td>13.6%</td>
<td>0.928</td>
</tr>
<tr>
<td>Autoimmune disease</td>
<td>50.0%</td>
<td>47.0%</td>
<td>0.752</td>
</tr>
<tr>
<td>Chronic Inflammatory disease</td>
<td>50.0%</td>
<td>43.9%</td>
<td>0.527</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>0.0%</td>
<td>1.5%</td>
<td>0.402</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>10.9%</td>
<td>15.2%</td>
<td>0.753</td>
</tr>
</tbody>
</table>