Wednesday, August 3, 2016

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

Infectious Disease

B-047

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

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

B-048

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

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

Methods: A total of 108 (52 newly diagnosed active PTB and 46 healthy control) subjects were recruited from western Regional Tuberculosis Center, Pokhara, Nepal for this study with the mean age of 37.31±1.72 years. Anthropometric variables, Lipid profile, total protein, albumin, uric acid, glycolysed haemoglobin (HbA1c), C reactive protein (CRP), total peroxide, total antioxidant substance, oxidative stress status, PON1 arylesterase and paraoxonase activities were determined in control and PTB subjects. *PON1*, Q192R, L55M polymorphisms were also determined in both healthy controls and PTB patients

Results: Significant difference in BMI, SBP and DBP was observed between PTB and control subjects ($p<0.001$, $p<0.01$ and $p<0.01$, respectively). Total protein, albumin, TC and HDL were significantly lower in PTB subjects in comparision to healthy controls ($p<0.05$, $p<0.001$, $p<0.01$ and $p<0.01$, respectively). Although, HbA1c, TG and LDL levels were also found decreased, were not statistically significant. The level of CRP, uric acid and globulin were significantly increased in PTB patients ($p<0.01$, $p<0.01$, $p<0.001$, respectively). Serum PON1 arylesterase and paraoxonase activity and total antioxidant substance were significantly lower in patients than control ($p<0.05$, $p<0.01$, and $p<0.05$, respectively) while total peroxide level and oxidative stress level were significantly higher ($p<0.01$, $p<0.001$, respectively). In PTB patients oxidative stress level was significantly correlated with PON1 paraoxonase activity ($r= -0.390$, $p< 0.001$). A statistically significant difference in PON1 L55M polymorphism was found between the PTB patients and the control group ($p=0.05$), however, there was no statistically significant difference in PON1 Q192R polymorphism between control and patients group ($p>0.05$). **Conclusion:** Patients with active pulmonary tuberculosis are exposed to potent oxidative stress and have decreased PON1 activity. These predisposing factors might play a role in inflammation and the pathogenesis of atherosclerosis in PTB.

B-049

An Evaluation of Performance of the VITROS® Immunodiagnostic Products HIV Combo Assay at two European Trial Sites.

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

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

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

Results: The specificity of the VITROS HIV Combo Assay for the donor population was calculated as 99.84% (5069/5077) 95% exact CI (99.69-99.93 %). The specificity of the VITROS HIV Combo Assay for the clinical population was calculated as 100% (607/607) 95% exact CI (99.39 -100 %).

When used to test 20 commercially available seroconversion panels, the HIV Combo assay was first positive at the same panel member as a commercially available 4th generation assay for 17 of the 20 seroconversion panels and was positive one panel member earlier, for the remaining three panels.

All 34 of the fresh HIV positive samples tested were positive in the VITROS HIV Combo Assay*

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

*In Development

B-051

Performance of a Prototype *T. cruzi* Assay* on the VITROS® ECi/ECiQ and 3600 Immunodiagnostics Systems for the Detection of Chagas Disease

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Background / Objective:

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

Method:

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

Results:

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

Conclusion:

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

* Under Development

B-052

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

l. fan[1], r. astles[1], h. burkom[2]. *[1]CDC, ATLANTA, GA, [2]Johns Hopkins University, Baltimore, MD*

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

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

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

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

Table 1. Correlation coefficients (**bold font** for values ≥ 0.7) between weekly time series of CMS claims data and gold standard reference surveillance data (NREVSS)

State	CMS/gold standard corr. coeffs. based on weekly test count volumes			CMS/gold standard correlation coefficients based on diagnosis codes for three CMS data types, three case definitions								
				Carrier			OTP			MAX		
	Carrier	OTP	MAX	CD1	CD2	CD3	CD1	CD2	CD3	CD1	CD2	CD3
CA	0.64	**0.86**	**0.84**	0.51	0.51	0.48	**0.72**	**0.77**	0.69	**0.83**	**0.81**	**0.82**
CO	**0.77**	**0.76**	**0.76**	0.56	0.44	0.41	**0.74**	0.63	0.67	0.60	0.58	0.61
FL	**0.73**	**0.81**	**0.73**	0.6	0.57	0.53	**0.80**	**0.74**	**0.78**	**0.77**	**0.74**	**0.71**
IA	**0.85**	**0.84**	**0.75**	0.68	0.50	0.43	**0.78**	**0.71**	**0.73**	0.52	0.50	0.51
IL	**0.77**	**0.80**	0.48	0.26	0.14	0.14	**0.82**	0.67	**0.73**	0.59	0.50	0.56
MA	**0.75**	**0.78**	**0.74**	0.48	0.36	0.35	0.65	0.55	0.66	0.51	0.45	0.58
NY	**0.81**	**0.85**	**0.82**	0.47	0.39	0.34	**0.79**	**0.74**	**0.82**	**0.81**	**0.74**	**0.81**
PA	**0.85**	**0.86**	**0.88**	0.64	0.52	0.46	**0.84**	**0.83**	**0.82**	**0.81**	**0.81**	**0.82**
TX	**0.77**	**0.84**	**0.76**	**0.7**	0.59	0.56	**0.84**	**0.83**	**0.79**	**0.84**	**0.82**	**0.82**
WA	**0.84**	**0.85**	**0.79**	**0.73**	0.63	0.62	**0.8**	**0.77**	**0.83**	**0.78**	**0.75**	**0.77**
Overall	**0.76**	**0.77**	**0.74**	0.54	0.50	0.42	**0.71**	**0.71**	0.64	**0.79**	**0.76**	0.63

B-053

Simultaneous detection of zika, chikungunya and dengue viruses in EDTA-plasma samples by RT-qPCR: If their vector is versatile their detection assays also should be

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Background:

Currently, multiple arbovirus are circulating in Brazil: Zika, chikungunya and dengue. They have similar clinical pictures, which can lead to misdiagnosis based on clinical grounds. RNA detection tests such as the RT-qPCR can reliably and specifically distinguish the three viruses and the specific diagnosis can be important in anticipating, preventing, and managing complications. Thus, the aim of the present study was to validate a RT-qPCR assay for simultaneous detection of these 3 viruses.

Methods:

This validation enrolled 90 EDTA-plasma samples from the arbovirus laboratory routine, 20 positive for zika (RT-qPCR), 6 positive for chikungunya (RT-qPCR), 18 positive for dengue (NS-1) and 46 negative for all 3 viruses. Nucleic acids were extracted from 1mL of sample by using an automated DNA extractor. An in-vitro transcribed random RNA sequence, which is not found in the nature, was spiked into plasmas during the nucleic acids extraction to function as a process control. Primers/probes for chikungunya were specifically designed for this study. Primers/probes for zika and dengue were obtained from literature. Zika, chikungunya and dengue viruses were assessed simultaneously by RT-qPCR, but in independent reaction wells. The control RNA was co-amplified in all instances. The viral loads of specific samples were quantified against a serial dilution of synthetic ssDNA and the limits of detection of each assay were determined by probit regression analysis (serial dilutions of each viral material from ~500 to ~0.5 copies/mL). To investigate the precision of the assays, three samples at ~72, ~7.2 and ~0.72 copies/mL of each viruses were evaluated by using the CLSI EP12-A2 method during 5 days in quadruplicate by two operators. The assays accuracies were evaluated by the agreement of the proposed RT-PCR with NS-1 assay for dengue and a second set of primers/probes for chikungunya and zika.

Results:

The limits of detection were 26 copies/mL (95%CI 14-89 copies/mL) for zika, 23.5 copies/mL (95%CI 13-81 copies/mL) for chikungunya and 25.6 copies/mL (95%CI 14-85 copies/mL) for dengue. The ~72, ~7.2 and ~0.72 copies/mL samples yielded 18/20 (90%), 2/20 (10%) and 1/20 (5%) positive results for zika, 18/20 (90%), 4/20 (20%) and 0/20 (0%) positive results for chikungunya and 20/20 (100%), 16/20

(80%) and 0/20 (0%) positive results for dengue, respectively. The total, positive and negative agreements between compared methods were 95.5% (95%CI 89-98%), 90% (95%CI 70-97%) and 97.5% (95%CI 90-99%) for zika, 100% (95%CI 95-100%), 100% (95%CI 61-100%) and 100% (95%CI 95.6-100%) for chikungunya and 95.6% (95%CI 89-98%), 100% (95%CI 81.5-100%) and 94.5% (95%CI 86.7-97.8%) for dengue, respectively. No cross-reaction was observed.

Conclusion:

The proposed RT-PCR method for simultaneous detection of zika, chikungunya and dengue viruses is highly sensitive, all assays showed limit of detection below 50 copies/mL. Moreover, cut-off regions were characterized and acceptable precisions were observed for positive (~72 copies/mL and above) and negative (~0.72 copies/mL and below) results. Finally, the agreements with the comparative methods were very good, above 90% of concordance in all instances. The main drawback of the study was that only 6 chikungunya samples were available.

B-054

Prevalence and Genotype Distribution of Cervical Human Papillomavirus in Korean Women

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

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

Methods:A total of 18,815 health examinees who were selected for this study, who took both tests of cervical cytology and HPV genotyping using multiplex PCR for screening of cervical cancer in 16 health promotion centers of 13 cities in Korea. The twenty-eight HPV genotypes were divided into two categories; high-risk HPV (carcinogen such as HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 69, and probable carcinogen such as HPV-68, 26, 53, 66, 70, 73, and 82), and low-risk HPV (HPV-6,11, 40, 42, 43, 44, 54, and 61).

Results:The overall HPV prevalence was 27.8%, with 22.2% of high risk HPV (HR-HPV), and 11.4% of low-risk HPV (LR-HPV). Among HR-HPV genotypes, HPV-53 was the most frequent (3.8%), followed by HPV-52(3.2%), HPV-70(3.2%), HPV-68(3.0%), HPV-58(2.7%), and HPV-16(2.0%). The prevalence of overall HPV, and HR-HPV infection increased along with the severity of cervical cytological findings (*P* for trend <0.001). The proportion of HR-HPV in relation with the age and cervical cytological findings, showed U-shape curve, with being the highest at age below 30 year-old, declining gradually down to the bottom at the age between 50-59, and then increasing afterwards in ASCUS/LSIL (*P*=0.001).

Conclusion:The prevalence and distribution of HR-HPV were different according to the age and cervical cytological findings, which provides a genotypic support for more effective screening of cervical cancer and appropriate vaccination.

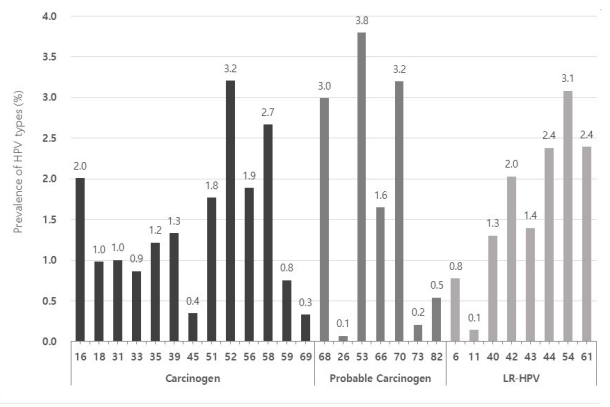

Fig. 1. Prevalence of HPV types in 18,815 Korean women.

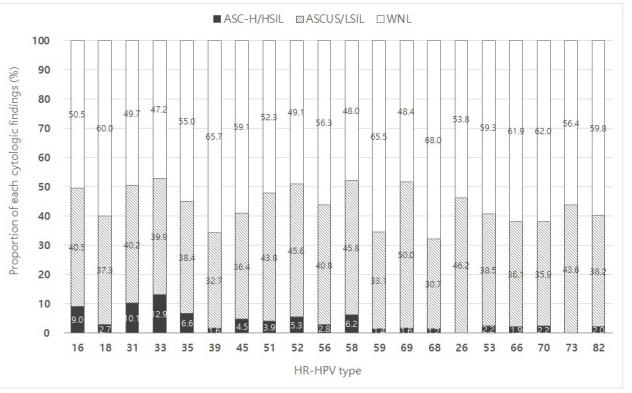

Fig. 2. Proportions of cervical cytology findings in each HR-HPV type.

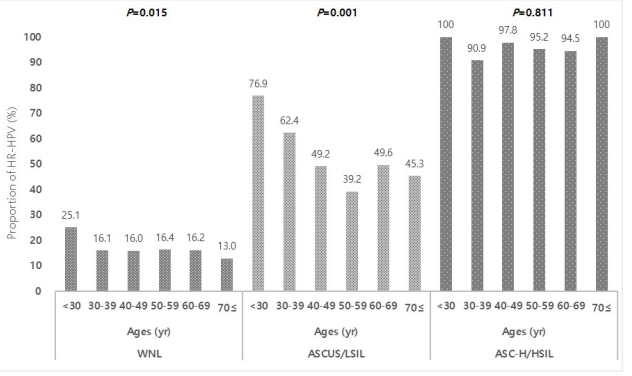

Fig. 3. Proportions of HR-HPV according to age groups and cervical cytology findings.
P for trend derived from Mantel-Haenszel test.

B-055

HIV-1 and HCV sequence-based genotyping methods validated against TRUGENE commercial kits.

N. d'Empaire, R. Guevara. *Biocollections Worldwide Inc., Miami, FL*

Background: The HCV and HIV-1 genotype determination have clinical importance because the response to antiviral treatment varies with the genotype. Furthermore, the classification of HIV-1 subtype and HCV genotypes provides a scientific opportunity to study the worldwide spread of these viruses. Sequence-based genotyping is the method that is most widely used to genotype. Some laboratories use in-house sequencing, but a number of commercial assays are also extensive used. **Methods:** Two genotyping assays were validated: one for HIV-1 and another for HCV genotyping in human plasma samples. For both assays, the viral RNA was extracted using the Abbott molecular m2000 sample preparation instrument. The HIV-1 genotyping assay

included two regions of the HIV-1 virus RNA: *env* gp41 immunodominant and *pol* IN. The sequences were amplified by RT-PCR using specific primers for each one. The *env* gp41 immunodominant region was amplified using the forward primer, JH35F, and the reverse primer, JH38R. The sequences from the *pol* IN region were obtained using primers poli5_OF and poli8_OR, followed by a nested PCR using primers poli7_IF and poli6_IR. Samples with low viral loads (< 500 copies/ml) were analyzed using an alternative protocol designed to amplify the *env* gp41 immunodominant region. Reverse transcription and primary PCR were performed using primers GP40F1 and GP41R1. Primers GP46F2 and GP47R2 were then used for the nested PCR. The HCV genotyping assay was carried out using sequences obtained from the NS5b region. The sequences were amplified by RT-PCR using the specific primers Pr1 and Pr2. Alternatively a hemi-nested PCR was carried out using primers Pr3, Pr4 and Pr5. The obtained sequences were blasted against available HIV and HCV sequence databases. **Results:** Results were validated against previously obtained results from the TRUGENE HIV-1 genotyping kit and TRUGENE HCV 5'NC genotyping kit. All 20 samples (100%) for the HIV-1 subtypes showed concordant results in both methods. However, at sub-subtype level, one sample was not coincident showing an F sub-subtype with TRUGENE method and an F2 sub-subtype in this study. The HCV genotypes determined by the two methods were concordant in 100% (21/21) of the samples but the results differed at subtype level (15/21). On the other hand, the NS5b method was able to classify all samples at subtype level whereas the TRUGENE HCV 5'NC genotyping kit did not in four cases (20%). **Conclusion:** It is concluded that these methods are reliable and convenient for HIV-1 and HCV genotyping. Moreover, the HIV-1 genotyping assay permitted subtype identification in samples with a low viral load and the HCV genotyping assay showed more resolution at the subtype level in comparison with TRUGENE methods.

B-056

Clinical application of a rapid POC immunoassay for Serum Amyloid A (SAA)

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Background:

Serum amyloid A (SAA), an apolipoprotein of high density lipoprotein (HDL) particles, is the precursor of AA fibrils in reactive amyloidosis. It is a sensitive acute-phase protein, which has been shown to have diagnostic utility in infections, unstable angina and in pediatric diseases. To permit early detection of these conditions, a rapid POC assay for the determination of SAA in plasma or whole blood samples was developed. The objective of this study is to demonstrate the clinical utility of the SAA determination using S-SPOT (Upper Biotech, Shanghai, PRC) which provides results in three minutes.

Methods:

Clinical samples were analyzed in parallel by both S-SPOT and a commercially available latex-enhanced rate nephelometry immunoassay for SAA, using samples from both healthy adults and patients. The S-SPOT method was calibrated using the WHO standard (92/680). Whole blood samples were measured on S-SPOT. Plasma samples derived from these specimens were re-assayed on S-SPOT to determine the impact of using whole blood as a sample material on S-SPOT. Samples from children with bacterial infection (n = 386) and with a viral infection (n = 219) were also assayed.

Results:

a. Healthy Subjects

The median of serum SAA from 240 normal healthy adults was 5.7mg/L, and the 95th percentile was 0-9.6mg/L.

b. Serum samples from patients

SAA values for patients ranged from 5 to over 1,700mg/L on S-SPOT. The study showed very good correlation between the S-SPOT and a commercially-available SAA test. A linear regression analysis showed y (S-SPOT) =1.134x+1.836; r^2=0.961, n=118.

c. Whole blood vs Plasma

The results obtained using whole blood correlated well with those obtained on the corresponding plasma samples. Linear regression: y (Plasma) =1.096x-2.704; r^2 = 0.976, n = 41.

d. SAA and CRP

SAA values for the pediatric patients were significantly increased compared to the values found for the classic and highly sensitive acute-phase reactant, C-reactive protein (CRP). The SAA values in patients with viral infections (Influenza virus, respiratory syncytial virus, adenovirus, etc.) were significantly higher than those in the control group ($P<0.05$) while the CRP in these patients showed no significant difference ($P>0.05$).

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

Conclusion:

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

B-057

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

A. L. Livermore, L. A. Brunelle. *Mayo Medical Laboratories New England, Andover, MA*

Objective:

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

Methodology:

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

The EUROIMMUN EUROLINE-WB IgG and IgM assays (EUROIMMUN AG, Luebeck, Germany) contain test strips with antigen extracts of B. burgdorferi. Test strips also contain a membrane chip coated with B31 antigens and recombinant VlsE antigen (IgG). Antibodies present in the patient serum bind to the antigens on the strip and are detected using an enzyme-labeled anti-human IgG/IgM which in the presence of substrate results in color development.

The Viramed Biotech IgG and IgM ViraStripe assays (Viramed Biotech AG, Planegg/Steinkerchen, Germany) contain specific B31 antigens that are bound to a solid phase nitrocellulose support membrane. Antibodies present in the patient serum bind to the antigens on the strip. Enzyme labeled anti-human IgG/IgM is added to detect bound antibody. Substrate is added which undergoes a color change.

Results:

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

Conclusion:

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

B-059

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

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Cervical carcinoma is the third most common cancer among women worldwide and most cases in developing countries. The carcinogenic human papillomavirus (HPV), a highly prevalent sexually transmitted infection (STI), is a necessary cause for the development of cervical intraepithelial neoplasia (CIN) and cervical carcinoma. However, among women infected with high-risk HPV, only some will develop CIN/ cervical carcinoma, suggesting that carcinogenic process following HPV infection and CIN is likely influenced by biological cofactors, such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum*, *Mycoplasma hominis,* and *Mycoplasma genitalium* infection that are often asymptomatic infections. Epidemiological studies about the impact of these infections on HPV acquisition and development to CIN or cervical carcinoma have yielded equivocal results due to the difficulty in separating biological from behavioral effects. The aim of this study was to investigate the incidence of HPV, *C. trachomatis*, *N. gonorrhoeae*, *U. urealyticum*, *M. hominis,* and *M. genitalium* in sexually active women and to evaluate the association these infections with CIN. A total of 126 patients was subjected to collection of cervical cytology. The samples (vaginal swabs) were examined by Real Time PCR and TaqMan detection system with specific primers and probes for each infectious agent. For the statistical analysis, the chi-square test was used considering the 0.05 significance level, and a multivariate analysis was performed by logistic regression. The results obtained showed that incidence of STIs was HPV 46.8%, *C. trachomatis* 27.8%, *M. genitalium* 28.6%, *M. hominis* 0.8%, *U. urealyticum* 4.8%, *N. gonorrhoeae* 4.8%. HPV (p= 0.024), *C. trachomatis* (p= 0.009), *M. genitalium* (p= 0.040) infection. Coinfection with HPV and *C. trachomatis* (p= 0.023), and HPV infection associated with the presence of at least one STI (p= 0.011) were associated with CIN. After multivariate analysis, a positive association was found between HPV and CIN (p= 0.040, OR= 2.48, CI: 1.04-5.92) and between *C. trachomatis* and CIN (p= 0.028, OR= 2.69, CI: 1.11-6.53). Therefore, in our study HPV, *Chlamydia*, *M. genitalium* infection and coinfection with HPV and *Chlamydia* and HPV infection associated with the presence of at least one STI constitute significant risk factors for the occurrence of CIN. Although these findings did not significantly differ by HPV genotype, the high frequency of these infections in asymptomatic women confirms that the inclusion of a diagnostic screening for detection of HPV in patients with STIs seems to be relevant in clinical and laboratory routine.

B-060

Comprehensive Clinical Evaluation of BacT Alert 3D Resin Bottles for Blood Cultures

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

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

Results: Of 2,994 sets received, 371 sets (12.4%) yielded 385 clinically significant pathogens. There was no statistically significant difference between prior antibiotic users (13.1%, 65/498) and antibiotics naïve patients (12.3%, 306/2,496) (P>0.05) in the positivity. Growth was observed in 0.35% (9/2,623 sets) in the terminal subculture. *Staphyloccus aureus*, gram-positive cocci, and all microorganisms were detected significantly faster in FA Plus (13.81 h, 14.09 h, and 11.12 h, respectively) than in FN Plus (14.82 h, 15.60 h, and 11.88 h, respectively). Otherwise, there was no significant difference in TTD for Gram-negative rod and yeasts. The distribution of the TTD was noteworthy. Over 88% of positive signals were encountered in the first 24 h. More than 97% were detected in both FA and FN bottles within 48 h. Within 72 h, 99.7% were detected in FA Plus and 99.0% in FN Plus.

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

B-061

Evaluation of Performance of Enzyme Linked Fluorescent Assay, Multiplex PCR and Real time PCR for Detection of Clostridium difficile

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

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

Results: Among 354 stool specimens, 38 (11.4%) were positive by VIDAS assay, 60 (16.9%) by PCR, and 82 (23.2%) by real time PCR. In comparison of real time PCR with VIDAS, positive concordance rate was 100%, negative concordance rate was 89.1% (P<.001), and discordance rate was 9.7%. Between PCR and VIDAS results, positive concordance rate was 84.2%, negative concordance rate was 93.4% (P<.001), and discordance rate was 7.6%. When real time PCR was compared with PCR, positive concordance rate was 93.3%, negative concordance rate was 91.2% (P<.001), and discordance rate was 8.5%.

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

B-062

Quantification of CSF chemokines and cytokines allows for rapid laboratory detection of CNS infections and further discrimination between viral and non-viral pathogens

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

into the CSF. Our objective is to use pathogen-specific chemokine/cytokine profiles to classify CNS disease as infectious versus non-infectious and further discriminate between viral and non-viral infections. **Methods:** Levels (pg/ml) of chemokines and cytokines were determined in the CSF of 45 patients with documented infectious meningitis or meningoencephalitis (mean age 19.2 years) and in the CSF of 25 patients who were negative for CNS infection (mean age 27.4 years). MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panels (Millipore) were used to measure CSF chemokines and cytokines levels (pg/ml). Innate immune analytes quantified included IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L. Samples were analyzed in duplicate by a FlexMAP 3D (Luminex). Standard curves were generated for each cytokine and median fluorescent intensities were transformed into concentrations by 5-point, non-linear regression. For univariate analysis, comparisons between groups were made using the Mann-Whitney test. We utilized receiver operating characteristic (ROC) curve analysis to calculate areas under the ROC curve (AUC) for each analyte to access the utility of chemokine/cytokine levels as discriminating tests. The ROC generated sensitivity and specificity values were then used to determine clinically optimal cutoff values for the informative analytes. **Results:** Univariate analysis utilizing Mann-Whitney tests demonstrated that median values (pg/ml) of IP-10 (CXCL10), IFNγ, IL-15, MDC (CCL22), MDC (CCL22), MCP-1 (CCL2), Fractalkine, and FLT3L were all significantly higher in CSF from patients with infectious brain disorders than in CSF from patients with non-infectious disorders (p-value < 0.05). MDC (CCL22) demonstrated statistical significance, when comparing viral infections versus non-viral infections (with the non-viral infection group having higher analyte levels). IP10 (CXCL10) can reliably distinguish between an infectious versus non-infectious CNS process (AUC 0.9778) with an optimal cut-off value of 2023 pg/ml (sensitivity, specificity; 93.0%, 92.0%). In the infectious group, MDC (CCL22) can reliably differentiate between viral and non-viral CNS infection (AUC 0.9545) with an optimal cut-off value of 194 pg/ml (sensitivity, specificity; 91.67%, 87.88%). **Conclusion**: CSF levels (pg/ml) of IP-10 (CXCL10) can reliably distinguish infectious versus non-infectious CNS disorders, and in the infectious group, MDC (CCL22) can reliably distinguish between viral and non-viral CNS infections. These results suggest that CSF chemokine/cytokine quantification can serve as a useful laboratory tool for the rapid triage of CNS diseases to help guide prompt therapy and further testing.

B-063

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

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

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

Results: All 111 HIV-1 positive samples were tested on both systems. 97.3% (108/111) samples were subtyped as CRF01_AE. In total, 647 DRMs were detected (435 in the RT gene, 199 in the PR gene and 13 in the Integrase gene). The *Sentosa* SQ HIV Genotyping Assay detected 100% (199/199) of all DRMs in the PR gene and more that 98% DRMs (427/435) in the RT gene. The Integrase gene was not included into the comparison study because it is not covered by the TruGene test. In total, 130 DRMs were detected by the *Sentosa* SQ HIV Genotyping Assay, that were not found by TruGene and 8 DRMs were missed by the *Sentosa* HIV Genotyping Assay (but detected by TruGene). Mutation detection rate for the HIV PR gene was 100% (95%CI: 98.11-100%) for the *Sentosa* SQ HIV Genotyping Assay and 90.45% (95%CI: 85.57-93.80%) for the TruGene system. In the RT gene 98.16% (95%CI: 96.41-99.07%) of DRMs were recorded by the *Sentosa* SQ HIV Genotyping Assay and 74.48% (95%CI: 70.18-78.35%) by TruGene. Overall DRM detection rates aggregated were 98.74% (95%CI: 97.53-99.36%) for the *Sentosa* SQ HIV Genotyping Assay and 79.5% (95%CI: 79.02-79.62%) for the TruGene HIV-1 Genotyping Kit. All HIV strains were carrying 1 or multiple DRMs in 61, 16 and 9 AA positions of the RT, PR and Integrase genes respectively. The most prevalent DRMs in the RT gene were: M184V was present in 48,7% (54/111) of the samples, K103N in 29.7% (33/11), Y181C in 27,9% (31/111), G190A and D67N (both 18.9% (21/111)). In the PR gene: M36I 91.9% (102/111), K20R 21.6% (24/111) and L10I 20.7% (23/111).

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

B-064

HCV Genotyping and Resistance-Associated Variants Detection Using Next-Generation Sequencing

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Background: Both conventional interferon based regimens as well as the increasingly used direct acting antivirals (DAAs) provide current treatment options for HCV. Accurate genotyping remains a prerequisite for IFN based treatment. However, with regard to DAAs one of the most important considerations is the potential development of Resistance-Associated Variants (RAVs) that may negatively affect sustained virological response. Timely detection and reporting of RAVs and HCV genotypes (GTs) is critical for drug regiment and can minimize the development of resistance to antiviral drugs. In this study we investigated the frequency of RAVs across HCV GT1, which is highly prevalent across geographic regions.

Methods: 346 prospective and retrospective EDTA-plasma and clinical serum samples from patents with chronic HCV infection across all 6 HCV GTs were tested in this study. We used a line probe-based test (VERSANT HCV Genotype 2.0 LiPA) in conjunction with the AutoBlot 3000H platform (SIEMENS) and a novel automated Next Generation Sequencing (NGS)-based integrated workflow, comprised of 1) a customized version of the epMotion 5075 (Eppendorf) robotic liquid handling system for RNA extraction and NGS library preparation (*Sentosa* SX101); 2) Ion Torrent technology for deep sequencing; 3) kits for nucleic acid extraction and NGS library preparation (*Sentosa* SQ HCV Genotyping Assay) and deep sequencing, respectively, and 4) data analysis and reporting software. The data reports on GTs 1a and 1b include 136 known RAVs in the NS3, NS5A and NS5B genes. Sanger sequencing was used as a reference method for all discordant and indeterminate samples.

Results: All 346 samples were tested on both platforms. For 47/346 (13.6%) samples GT results by VERSANT were "indeterminate". In 19/299 (6.4%) of the samples, discordant results between the two methods were obtained. The ability to correctly determine HCV genotypes was 93.7% (95%CI: 90.3-95.9%) for VERSANT and 100% (95%CI: 98.7-100%) for *Sentosa* HCV Assay. Sanger sequencing confirmed that all 19 discordant samples were incorrectly classified by line probing. GT distribution among the 47 samples indeterminate by VERSANT was: 5 GT1a, 1 GT2, 19 GT3, 1 GT4, 20 GT6 and 1 mixed infection (GT2 and GT3). Clinical sensitivity aggregated was 86.4% (95%CI: 82.4-89.6%) for VERSANT and 100% (95%CI: 98.9-100%) for *Sentosa* HCV. 56 GT1a and 54 GT1b samples were used for further analysis of RAVs distribution among the GT1 population. 52.7%(58/110) of HCV strains were carrying 1 or multiple RAVs in 23 positions across all target genes. An unequal distribution of 4 mutations across the GT1 subtypes was observed. Frequency of the Q80K mutation (NS3) was 25%(14/56) in GT1a and 1.9%(1/56) in GT1b. While mutations Q54H and Y93H (NS5A) were prevalent in GT1b: 42.6%(23/56) and 18.5%(10/56) respectively. No Q54H mutation was present the GT1a population studies; Y93H in this group reached 1.8%(1/56). Mutation V499A in the NS5B gene was present in the GT1b population at 25.9%(14/54) and absent in the GT1a population.

Conclusion: Simultaneous determination of HCV genotypes and detection of RAVs in single NGS runs provides comprehensive, clinically relevant information for optimal selection of HCV treatment regimens.

B-065

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

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

Methods: In this prospective study, 1087 consecutive urogenital samples from symptomatic and asymptomatic patients were evaluated with the PlexPCR *M. genitalium* ResistancePlus kit. This is a real-time PCR kit that employs novel PlexPrimer and PlexZyme technology. PlexPrimers selectively amplify mutants over wild-type and PlexZymes allow for efficient multiplexed detection and signalling. This unique combination allows "stacking" of the 5 mutation assays for a single readout. This kit was run in parrellel to an in-house test for Mg detection and sequencing of Mg positives to determine 23S rRNA mutation status.

Results: The prevalence of Mg was 6.0% (65/1087) and in the Mg positive samples 23S rRNA mutation prevalence was 63.1% (41/65). The PlexPCR *M. genitalium* ResistancePlus assay showed very high clinical performance compared to the reference methods with sensitivity and specificity for Mg detection of 98.5% (95%CI: 91.7-100%) and 100.0% (95%CI: 99.6-100%), and 23S rRNA mutation detection of 92.7% (95%CI: 80.1-98.5%) and 95.7% (95%CI: 78.1-99.9%) respectively. The PlexPCR *M. genitalium* ResistancePlus assay showed a limit of detection (LOD) of 10 - 15 copies for each mutant when LOD study was performed on synthetic template and no cross-reactivity of related organisms was seen.

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

B-066

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

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

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

Results: Evaluation of the patient samples using the ADVIA Centaur CMVIgG assay indicated that the positive agreement ranged from 98.7% to 99.4% and the negative agreement ranged from 98.5% to 99.5%, when compared to the VIDAS CMV IgG assay. Additionally, the ADVIA Centaur CMVIgG assay displayed a total agreement of 99.3% (133/134) to the resolved clinical status of all CMV IgG-negative cross-reactive samples evaluated. The assay demonstrated good precision, with an average repeatability and within-run %CV of <5.0% and <9%, respectively, for samples yielding Index values between 0.50 and 30.00.

Conclusion: The results of this study demonstrate good performance of the prototype ADVIA Centaur CMVIgG assay.

*For investigational use only. Not available for sale. The performance characteristics of this product have not been established.

B-067

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

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

B-068

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

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Background: Sexually transmitted infections (STIs) present a major public health concern worldwide. Reduction of complication rates and disease spread requires the use of rapid and reliable laboratory diagnostic tool that can identify causative pathogen. Multiplex real-time PCR assay is a highly sensitive and effective diagnostic tool for detection of urogenital pathogens, especially for culture-difficult organisms. The objective of this study was to assess the prevalence of six common sexually transmitted pathogens and the co-infection rate by multiple pathogens in female genital tract using a multiplex real-time PCR assay. **Methods:** The authors retrospectively analyzed the STIs multiplex real-time PCR results from 3,460 women (mean age: 36.7±10.1 years). STIs tests were requested to Seoul Medical Science Institute from 43 medical institutions nationwide in Korea between January 2015 and September 2015. Multiplex real-time PCR assays were performed with cervico-vaginal swabs by use of *AccuPower®* STI8A-Plex Real-Time PCR Kit (BIONEER, Korea). Detection of the six types of STI pathogens including *Micoplasma hominis* (MH), *Ureaplasma urelyticum* (UU), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Trichomonas vaginalis* (TV), and *Neisseria gonorrhoeae* (NG) was performed simultaneously in a single multiplex real-time-PCR reaction using a combination of primers for each pathogen. **Results:** Of the 3,460 subjects, 1,266 (36.6%) tested positive for at least 1 pathogen, with 836 (24.2%) positive for 1 pathogen, 337 (9.7%) for 2 pathogens, 87 (2.5%) for 3 pathogens, and 7 (0.2%) for 4 pathogens (n =1,799 pathogens total). Among the 836 subjects infected by single pathogen, the prevalence of each microorganisms were as follows; 349 tested positive for MH (41.7%), 337 for UU (40.3%), 68 for CT (8.1%), 46 for MG (5.5%), 25 for TV (3.0%), and 11 for NG (1.3%). Among the subjects infected by 2 pathogens, co-infections with MH and UU were most frequently observed (185/337, 54.9%). Other combinations of infection by 2 pathogens were as follows; MH and CT, 44/337 (13.1%): MH and MG, 24/337 (7.1%): MH and TV, 23/337 (6.8%): UU and CT, 23/337 (6.8%): CT and MG, 9/337 (2.7%): UU and MG, 7/337 (2.1%): UU and TV, 6/337 (1.8%): MH and NG, 5/337 (1.5%): UU and NG, 4/337 (1.2%): CT and NG, 3/337 (0.9%): CT and TV, 2/337 (0.6%): MG and TV, 2/337 (0.6%). Among the subjects infected by 3 pathogens, con-infections with MH, UU, and CT (20/87, 23.0%) were the most frequent. **Conclusion:** STIs are prevalent in sexually active women in Korea, and infections by MH and UU are especially frequent. As the proportion of co-infection by multiple pathogens was noted up to 34.0% among the STIs-positive subjects, guidelines establishment regarding screening of STIs by multiplex PCR approach in high risk group is needed. Correct diagnosis of STIs by multiplex real-time PCR apprach will guide the appropriate interventions targeted against specific pathogens. Effective treatment of STIs in sexually active women has important implications for the reduction of side effects and risk to fetal development, and for the prevention of onward transmission.

B-069

The utility of QuantiFERON-Gold in Tube assay to diagnosis of active tuberculosis: Using new equation

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Objective

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

Method

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

Results

The number of active TB and non-TB was 144 (age 51.5±19.0, M:F=86:58) and 151 (age 63.5±14.2, M:F=98:53), respectively. The INF-γ levels of NIL and Mitogen tube were inversely correlated with age but not in TBAg tube. The active TB showed higher IFN-γ levels than non-TB in Nil tube (0.24±0.24 vs. 0.11±0.16, $P<0.001$) and in TBAg tube (4.84±3.45 vs. 3.19±2.93, $P<0.001$). But there was no difference in IFN-γ levels of Mitogen tube between TB and non-TB. The cut-off of INF-γ in Nil and TBAg were >0.08 (AUC 0.72, sensitivity 72%, specificity 64%) and >2.83 (AUC 0.65, sensitivity 63%, specificity 64%). Newly derived equation maximizing the discrimination power was $(Nil \times 10)^2 (TBAg - Nil)$. The AUC of new equation was 0.75 (cut-off >4.42, sensitivity 59%, specificity 80%). The combination of new equation and Nil for active TB diagnosis showed sensitivity 57% and specificity 80%.

Conclusion

The QFT-GIT assay is in vitro test with high sensitivity and low positive predictive value. To help to find real active TB infection among positive QFT-GIT cases, this study suggests a new equation. The diagnostic value of new equation was better than individual IFN-γ levels of QFT-GIT. TB suspects who present with stronger IFN-γ responses in Nil tube and TBAg tube and high calculated value through the new equation should be carefully evaluated to exclude the possibilities of active TB infection in TB-endemic area. To accurate differential diagnosis of active TB using IGRAs more clinical researches involving the IGRAs of new formats are needed. New format of IGRAs should have additional cytokines or diagnostic formula for discrimination of active TB infection.

B-071

Prevalence of HIV-1 genetic/recombinant subtypes and drug resistance mutations in Brazil, updated 2015

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Background: Genetic variability is a major feature of the human immunodeficiency virus type 1 (HIV-1). This viral diversity has impact on the diagnosis, monitoring, therapy and development vaccines. Subtyping is a powerful molecular tool for the choice of an effective therapy and monitoring the evolution of the HIV-1 epidemic. In Brazil, the majorities of published data about the prevalence of HIV-1 are from south and southeast remain scarce in other regions. **Objective:** To estimate the prevalence of HIV-1 subtypes and resistance profiles in order to contribute to the implementation of public health *policies in Brazil.* **Method**: Plasma samples from 184 diagnosed HIV-infected patients of different geographical regions of Brazil with viral loads above 1,000 copies/mL were used. Viral cDNA fragments corresponding to the reverse transcriptase and protease regions were amplified and sequenced. The subtype and mutations profile were assessed using Stanford HIV Drug Resistance Database. **Results:** The mean age of the patients was 37.17 (range 4-74 years), and most samples were from men (80.43%). Among the 184 patients, 144 (78.26%) presented mutation in the analyzed region (21.74%), of which 96 (52.17%) to NRTIs, 104 (56.52%) toNNRTIs and 82 (44.57%) to protease inhibitors. According to analyses, a variety of subtypes and recombinant forms were detected (Figure 1A), the most frequently been the subtype B. The viral recombinant forms was identified at 17 patients, and seven different types of recombinant were recognized(Figure 1B). The distribution of HIV-1 subtypes and recombinants within each region in Brazil is revealed in Figure 1C. **Conclusion:** The maintenance of HIV-1 genotyping programs is important in the management of patients for first line and rescue therapy, and the attempted monitoring of the HIV-1 subtype prevalence in Brazil. Furthermore, these surveys produce data that should be an important resource for all HIV scientists and public health officials.

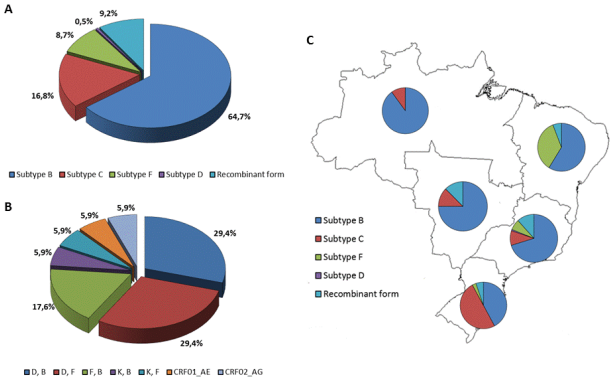

Figure 1: (A) Frequency of HIV-1 subtypes and recombinant forms identified at present study. (B) Frequency of different types of recombinant form. (C) Recent geographical distribution of HIV-1 subtypes and recombinants in Brazil.

B-072

Influenza Rapid Testing Course and New Influenza Pandemic Preparedness Course Offered for Clinicians - Utilization of Courses and E-Resources

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Objective: To enhance the knowledge of clinicians when using rapid influenza diagnostic tests (RIDTs) by offering a free online influenza rapid testing course, "Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS)", with continuing education credits (CEs). The course focuses upon RIDT utilization. Further objectives were to gather feedback from course participants about SIRAS and about other educational needs, and based on that feedback, to develop and offer a new online influenza pandemic preparedness course with CEs. Data on course utilization and on utilization of associated e-resources are presented. **Relevance:** RIDTs are often used for making treatment decisions for patients suspected of having influenza. Concerns persist about the variable predictive value of RIDTs, within the context of the prevalence of circulating influenza viruses in the population being tested. This is of particular concern in outpatient and ambulatory settings where RIDTs are often the only readily-available influenza tests. **Methods**: The SIRAS course was originally offered in Oct. 2012, and restructured in 2013 from a single to four 30- minute modules (http://www.jointcommission.org/siras.aspx). The new influenza pandemic preparedness course, "Influenza Preparedness and Response in Ambulatory Settings" (http://www.jointcommission.org/topics/influenza_pandemic_preparedness.aspx) was developed and launched in Apr. 2015 and restructured in Oct. 2015 into two shorter segments: 1) pandemic planning, the response phase and 2) laboratory testing/diagnosis and patient management/treatment. Both courses were developed with a technical panel of influenza experts. Specimen collection videos https://www.youtube.com/playlist?list=PLNQfL_CJ36fK08KEPjxu1ZKJn7GuFtn-N. , and other e-resources and helpful links (such as CDC FluView) are offered with the courses. Courses are updated annually and relaunched in Oct. of the respective year. Multiple social media and Joint Commission communication channels are used for marketing. Course utilization data and e-resource utilization data are tracked. The SIRAS course is currently being translated into Spanish. **Validation**: There were 5,864 unique visitors to the SIRAS webpage and 1,615 enrollments in SIRAS between Oct. 2014 and Sept. 2015. There were 1,722 visitors to the pandemic course webpage and 608 course enrollments since its original launch in Apr. 2015 through Sept. 2015. Since updating and relaunch of both courses in Oct. 2015, there have been 458 enrollments in SIRAS and 301 enrollments in the pandemic course, representing upward trends. The SIRAS certificate issuance rate has increased to 82% (Oct. - Dec. 2015) compared with 34% during the first year of offering and 48% last year. There were 2,345 downloads of the Infographic describing the courses; 10,162 views of the RIDT post on The Joint Commission's "AmBuzz" blog, and 132,667 views of the specimen collection videos, cumulatively from the initial offering through Nov. 2015. The overall satisfaction rates were 99% for the SIRAS course and 94% for the pandemic course. **Conclusions**: Ambulatory care providers have welcomed the ongoing opportunity for continued education in influenza testing and preparedness. The annual updating of all course modules before the onset of influenza season specifically attracts high usage of the e-resources and increased course enrollments. YouTube specimen collection videos, and other course e-resources are increasingly popular.

B-073

Distribution and Phenotypic Resistance Profile from Bloodstream Infections Isolates from Metropolitan Public Hospitals of Sao Paulo City, Brazil: Data from a Laboratory of Clinical Microbiology

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Background: Nosocomial Bloodstream Infections (BSI) are directly related with high morbidity and mortality rates, hospital lengths of stay in critical ill patients mostly and associated health care costs. Brazilian studies concerning Bloodstream Infections are important tools that can bring specific issues related to antimicrobial resistance. The aim of this study was evaluate the frequency of microorganism isolation and the main phenotypic resistance profiles from BSI in medical centers located throughout in the city of Sao Paulo, Brazil. **Methods:** Data on bacterial and fungal isolates from BSI patients admitted to the 10 public health system medical centers were selected from July 2014 to July 2015. Identification and susceptibility tests were performed using Vitek MS and Vitek 2 (bioMerieux) systems, respectively. Antimicrobial susceptibility profile were evaluated according CLSI, 2014/2015 criteria. Coagulase Negative Staphylococcal (CNS) BSI was only considered in the presence of at least two sets of positive blood cultures with the same organism. **Results:** A total of 8632 microorganisms were evaluated. The frequency of organism group types were Gram-positive (n=4841; 56.1%), Gram-negative (n=3359; 38.9%), yeasts (n=400; 4.6%) (1.5% *Candida albicans*; 2.9% *C.* non *albicans* and 0.2% others yeasts) and 0.4% fastidious organisms (*Moraxella* spp.; *Neisseria* spp. and *Haemophilus* spp.). The most frequent organisms were CNS (26.7%); *Staphylococcus aureus* (17.1%); *Klebsiella pneumoniae* (10.7%); *Acinetobacter baumannii* (7.2%); *Escherichia coli* (6.4%); *Enterococcus* spp. (5.9%); *Pseudomonas aeruginosa* (3.4%); *Candida* non *albicans* (2.9%); *Streptococcus viridans* group (2.9%) and others Gram-negative non-fermenter (2.8%). Antimicrobial resistance was observed mainly in 88.7% CNS (2050/2311) and 52% *S. aureus* (769/1479) to methicillin; and 39.2% (200/510) of *Enterococccus* spp. to vancomycin. Carbapenems resistance was detected in 78.1% (485/621) of *Acinetobacter* spp isolates, 43% (398/926) *K. pneumoniae*; 31.3% (92/294) *P. aeruginosa*; 4.7% (10/211) *Enterobacter* spp. and 2.3% (25/1066) of others Enterobacteriaceae. **Conclusion:** In contrast with other Brazilian studies, *P. aeruginosa* was not the main organism recovered from BSI, although the resistance rate to carbapenem was one of the most higher when compared with others Gram-negative. Rates of antimicrobials resistance reported in CNS, *Enterococcus* spp. and *K. pneumoniae* are reinforce the high resistance rates to antimicrobials in Sao Paulo city that is considered one of the highest observed in Brazil. A continued study is necessary to monitor these national trends.

B-074

Beckman Coulter DxN VERIS Molecular Diagnostic System Sample-To-Sample Crossover Contamination Study

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OBJECTIVE: Sample carry-over and cross-contamination present a high risk for diagnostic medical devices and therefore are monitored to determine their impact. Based on internal and external voice of customer surveys, minimal/no sample carryover and cross-contamination were recognized as critical system characteristics that had to be addressed in the development of a 21st century molecular diagnostic random access instrument, Beckman Coulter's DxN VERIS Molecular Diagnostic System*. However, there are no guidelines on the statistical methodology for data analysis, especially where the absence of carryover is necessary. Furthermore, there is a lack of criteria for analytical specificity that rigorously challenges the system being tested. The DxN VERIS was designed to have a false positive rate due to cross contamination of less than 1 in 500 tests with 95% confidence with an overall design goal of zero. The objective of this study was to assess the sample-to-sample contamination rate using a real-time PCR assay, characterize potential sources of contamination, and assess the effective resolution of carry-over and cross-contamination artifacts.

METHODS: Contamination characterization was performed by swabbing areas of the instrument before and after running a series of high concentration level positive samples to determine potential sources of contamination. Swab assessments identified areas where contamination of liquid handling was occurring on the instrument. Several modifications to the liquid handling parameters were incorporated into the test processing system to eliminate sample-to-sample contamination. The analytical specificity, as a measurement of cross-over contamination, was assessed using the optimized liquid handling parameters with the VERIS Hepatitis B (HBV) assay. In order to fully evaluate the ability to prevent instrument induced cross-contamination,

high positive HBV plasmid samples at a concentration of 1×10^9 IU/mL, which represents a concentration level exceeding 95% of the diseased population for any of the assays developed on DxN VERIS, were tested interspersed with true negative samples composed of sterile tris ethylenediaminetetraacetic acid (TE) buffer.

RESULTS: A total of 2,330 true negative samples were processed alternating with 2,330 high positive samples, providing the statistical power to evaluate the false positive rate using a lower bound two-sided 95% confidence interval. Because PCR can detect single molecules of nucleic acid, a 1 picoliter (1.0×10^{-12} liter) droplet size at a concentration of 1×10^9 IU/mL, would cause a false positive result. Based on these data, the DxN VERIS did not exhibit detectable carryover from the high positive samples to the negative samples and was verified to have a false positive rate due to cross contamination of 0% with a lower bound two-sided 95% confidence interval of 99.9%, providing evidence for the absence of sample-to-sample carryover.

CONCLUSIONS: The Beckman Coulter DxN VERIS Molecular Diagnostic System did not exhibit detectable carryover from high positive samples to negative samples. This was true when the concentration of target in the samples was significantly higher than target concentrations found in clinical samples.

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

B-075

Performance Evaluation of Prototype Herpes-I IgG and Herpes-II IgG Assays* on the ADVIA Centaur XP Immunoassay System

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

Methods: Positive percent agreement (PPA%) and negative percent agreement (NPA%) for the Siemens Herpes-I IgG and Herpes-II IgG assays were assessed by testing on 970 serum and 670 plasma samples against LIAISON (DiaSorin) and HerpeSelect 1 and 2 Immunoblot IgG (Focus) assays, respectively. Samples came from Banc de Sang i Teixits de Catalunya (Spain) (300 sera), from different commercial sources (75 samples), from sexually active adults provided by ProMedDx (Norton, U.S.) and Cerba (Spain) (367 sera), and from pregnant women, also acquired from ProMedDx (Norton, U.S.) (228 sera). Other relevant performance characteristics such as 20-day precision (CLSI EP5-A3), reagent opened onboard stability (CLSI 25-A), interferences (CLSI EP7-A2), and cross-reactivity were also determined.

Results: For the Herpes-I IgG assay, PPA% was 98.6% and 98.0% and NPA% was 95.3% and 95.3% against LIAISON and Immunoblot, respectively. For the Herpes-II IgG assay, PPA% was 97.3% and 96.5% and NPA% was 95.1% and 96.9% against LIAISON and Immunoblot, respectively. Within-run and total precision %CV was found to be between 1.7 and 5.1% and 3.1 and 6.6% for the Herpes-I IgG assay and between 1.2 and 2.0% and 2.8 and 4.1% for the Herpes-II IgG assay. Reagent opened onboard stability was verified up to 8 weeks for both assays. Additionally, for both assays, no interferences (hemoglobin, bilirubin, triglycerides, and cholesterol) or cross-reactivity were observed with 24 different disease conditions.

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

*Under development. Not available for sale.

B-076

Quantitative Detection of CMV, HBV, HCV, and HIV-1 on the Beckman Coulter DxN VERIS System

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

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

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

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

The Wilson Score method yielded 100% specificity with a lower bound confidence interval of 98.7% for CMV, 100% specificity with a lower bound confidence interval of 99.5% for HBV, 100% specificity with a lower bound confidence interval of 99.0% for HCV, and 99.8% specificity with a lower bound confidence interval of 99.1% for HIV-1 at the 1 mL sample volume assay.

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

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

B-077

Enabling Random Access Molecular Testing Using Multiple PCR Cells

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OBJECTIVE: Beckman Coulter's DxN VERIS Molecular Diagnostic System* is a fully automated sample-to-answer system for quantitative and qualitative analysis of molecular targets. The system integrates and automates all major processes, from sample introduction and nucleic acid extraction and purification, to real-time PCR amplification and detection. In order to meet the throughput goals of 150 tests per 8 hour shift and the random-access testing goal via a one-piece flow process, 20 PCR cells are required. The objective of this study is to assess the level of uniformity across the 20 PCR cells.

METHODS: A DxN VERIS system was used for testing. The 20 on-board PCR cells each accommodate a single 40 µL sample volume vessel. Sample heating is achieved by applying a voltage across heating tape thermally communicating with the reaction vessel. Sample cooling is achieved by flowing ambient temperature air across the reaction vessel. Laser-induced fluorescence is made using a dual laser excitation / CCD detection system.

To compare the sample temperature experienced across all 20 PCR cells, thermistors were placed in reaction vessels filled with 40 µL water. Thermocycling temperature profiles were collected near the extremes and in the middle of the full temperature range, namely 45°C, 70°C and 95°C, with dwell times of 25 seconds per temperature over 20 cycles.

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

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

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

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

B-078

Workflow Characteristics of the Beckman Coulter DxN VERIS Molecular Diagnostic System Compared Against Four Automated Laboratory Instruments

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OBJECTIVE: Automated molecular diagnostic instruments present standardized processing schemes and workload advantages to the clinical laboratory. Assessments of workflow and maintenance characteristics provide quantitative and objective metrics that accurately measure automated system efficiency and associated labor costs. The objective of these series of studies was to determine the workflow characteristics of the Beckman Coulter DxN VERIS Molecular Diagnostic System* evaluated against four existing batch and semi-automated platforms.

METHODS: Multi-site comparative workflow studies were conducted on the DxN VERIS and four other platforms by Nexus Global Solutions, Inc., an independent third party healthcare and diagnostic consulting firm. The targeted parameters of the study included system complexity (process and consumables), system operating and performance metrics, hands-on time requirements, and total process times for the HIV-1, HCV, HBV, and Cytomegalovirus (CMV) viral load assay workflows. Testing methodology and work practices were captured and documented through direct observations and practices, time study of operators and analytical systems, and targeted interviews with operators. Operational metrics were attained by means of process steps, value streams, operational complexities, testing timelines, and process times.

RESULTS: DxN VERIS had 10 process steps compared to 29 steps for Roche Cobas® Ampliprep/TaqMan®-48 (CAPTM-48) and Siemens kPCR (kPCR), 30 steps for Hamilton MagNA STARlet/Roche MagNA Pure-96/Roche LightCycler-480 (MS+MP+LC), and 26 steps for Abbott m2000 (M2000). DxN VERIS had five consumables required for sample processing. In contrast, kPCR and m2000 had >20 consumables, while all others had >10 consumables. DxN VERIS showed the shortest time to first result (TTFR) at 1.25 h for CMV and HBV and 1.75 h for HIV-1 and HCV, with subsequent results occurring every 2.5 min. DxN VERIS also showed a time to last result (TTLR) at 4.15 h for CMV and HBV (48 tests) and 4.65 h for HIV-1 and HCV (48 tests). The other platforms processed samples in batch mode, therefore having identical TTFR and TTLR. CAPTM-48 yielded a process time of 7.58 h for 48 HIV-1 samples and 7.2 h for 48 HCV samples. kPCR were 6.93 h for 96 HIV-1 Patient Samples. Process time for MS+MP+LC had 3.32 h for 48 HBV and/or CMV samples. m2000 had a process time of 6.42 h for 48 HIV-1/HCV samples, 5.43 h for 24 HBV samples, and 5.87 h for 24 CMV samples. DxN VERIS had a hands-on time requirement of 23 min for 144 patient samples. The hands-on time requirement processing 48 samples for CAPTM-48 was 60 min, MS+MP+LC was 84 min, and m2000 was 63 min. kPCR had a hands-on time requirement of 58 min for 96 samples.

CONCLUSIONS: Considerable time requirements and process complexity differences were observed between instruments. While in these particular studies MS+MP+LC showed the fastest TTLR of 3.32 h for the HBV and CMV assays, compared to the DxN VERIS system at 4.15 h, DxN VERIS was faster for the HCV and HIV-1 assays, had the shortest TTFR, the fewest process steps, the largest test capacity, and the lowest hands-on time requirements.

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

B-079

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

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

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

Results: The Lumipulse G TP-N demonstrated a 20-days total imprecision ≤ 5.3% (n = 80), site to site ≤ 5.4% (n = 120) and Lot to lot ≤ 6.7% (n =120) using 5 serum, one Na Citrate plasma panel and one commercially available control. One thousand seven hundred forty-five (1745) samples were used to compare the Lumipulse G TP-N with the Final Comparator Result using a Treponemal IgG/IgM test (Centaur ADVIA), Nontreponemal test (RPR), and 2nd Treponemal test (Serodia TP-PA). The Positive Predictive Agreement (PPA) was 96.8% and the Negative Predictive Agreement (NPA) was 98.0%.The PPA results of Lumipulse G TP-N for a total of 724 apparently healthy patients (apparently healthy adults, children and pregnant women) were 99.7%. Two samples tested positive (1 apparently healthy adult and 1 pregnant woman) for Lumipulse G TP-N. The apparently healthy adult was confirmed positive by Serodia TP-PA. The pregnant woman was negative per Serodia TP-PA. The Lumipulse G TP-N demonstrated an average interference of ≤ 10% (for each compound) in a study with human serum specimens supplemented with potentially interfering compounds individually at indicated concentrations: 1000 ng/mL HAMA, 1000 IU/mL rheumatoid factor, 40 mg/dL conjugated and free bilirubin, 3000 mg/dL triglycerides, 500 mg/dL hemoglobin, g/dL protein biotin 500 ng/mL, gamma globulin 60 mg/mL, cholesterol 400 mg/mL, and ascorbic acid 3 mg/dL.

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

B-082

The Diagnostic Accuracy of Various HIV Assays for Discrimination Between HIV-1 and HIV-2 Infections

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Background: Discrimination between HIV-1 and HIV-2 infections is crucial for selection of appropriate treatment options. For this reason, Centers for Disease Control and Prevention (CDC) has recently added HIV1/2 discrimination step in the recommended laboratory HIV testing algorithm. However, lack of experience and limited number of available assays still make it difficult to distinguish between two viruses. In addition, it is often very difficult to interpret the assay results due to strong antibody cross-reactivity. So, we evaluated diagnostic accuracies of several assays which are helpful in diagnosing and discriminating HIV1/2 infections.

Methods Between 2013 and 2015, total 57 plasma samples from HIV infected patients were collected from Togo and provided by Human Serum Bank. To distinguish between HIV-1 and HIV-2 infection, three rapid tests, five Western blot assays, and one line immunoassay were performed. We also performed real-time reverse transferase polymerase chain reaction (RT-PCR) for the confirmation of HIV-1 or HIV-2 infection.

Results: Of 57 samples we confirmed 25 samples as HIV-1 infection and 31 samples as HIV-2 infection, and one sample as dual infection. There were best diagnostic accuracies in SD Bioline HIV-1/2 3.0 for detection of HIV-1 (87.7%) and INNO-LIA HIV-I/II Score for detection of HIV-2 (96.4%). With dilution of samples to reduce the cross-reactivity between HIV-1 and HIV-2 antibodies, there was impressive improvement in diagnostic accuracy of SD Bioline HIV-1/2 3.0 (98.2% for HIV-1 and 100% for HIV-2). Meanwhile, the Western blots except MP HIV Blot 2.2 were little helpful for the discrimination of HIV types due to cross-reactivity and high rate of indeterminate results.

Conclusions: SD Bioline HIV-1/2 3.0 and INNO-LIA HIV-I/II Score showed best performance in accurate diagnosis of HIV-1 and HIV-2 infections. However, severe cross-reactivity was shown in Western blot assays except one MP HIV Blot 2.2, thus this resulted in difficulty of differentiation between two viruses. Therefore line immunoassay and rapid test would be the best option for discrimination between HIV1/2 infections.

Acknowledgement: This research was supported by a grant (14172MFDS234) from Ministry of Food and Drug Safety and Human Serum Bank.

B-084

Development of a highly sensitive Procalcitonin Assay for the LUMIPULSE® G1200 and G600II analyzers.

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Background: Procalcitonin (PCT), a precursor of calcitonin, is synthesized by C-cells in the thyroid under healthy conditions. However, systemic inflammatory responses triggered by severe bacterial infections or sepsis induce systemic synthesis of PCT, and then it results in elevated PCT level in blood. Therefore, the determination of PCT levels in blood can be used to formulate differential diagnosis or to indicate severity of severe bacterial infections and sepsis. We have developed new chemiluminescent enzyme immunoassay (CLEIA) for PCT using fully-automated LUMIPULSE G1200 and G600II systems. Here we report the fundamental performance of the PCT assay.

Methods: B•R•A•H•M•S PCT assay for LUMIPULSE G1200 and G600II systems is a two-step sandwich assay based on CLEIA technology. The resulting reaction signals are derived within 30 minutes/test, and are proportional to the amount of PCT in the sample allowing quantitative determination of serum or plasma PCT.

Results: The detection limit was 0.0047 ng/mL, and the limit of quantitation was 0.0079 ng/mL. A 20-day precision study was performed during a 31-day period using two controls and three panel specimens, and the imprecision was ≤ 4% total CV. Dilution linearity was evaluated using three test samples, and the recovery rate of up to 32-fold dilution was 90-102% for manual dilution and 99-104% for 10-fold automated dilution within the calibration range of 0.02–100 ng/mL. The correlation coefficient and the regression slope and Pearson's correlation coefficient of Lumipulse *G* B•R•A•H•M•S PCT and B•R•A•H•M•S PCT sensitive KRYPTOR, based on Time-Resolved Amplified Cryptate Emission (TRACE) Technology (Thermo Fisher Scientific), were 1.01 and 0.96, respectively (N=85). On the correlation between LUMIPULSE G1200 and G600II systems, the slope and correlation coefficient were 1.00 and 1.00, respectively. No interference was observed with unconjugated (20.0 mg/dL) or conjugated bilirubin (21.1 mg/dL), hemoglobin (294 mg/dL), triglyceride (2,000 mg/dL), and protein (4-12 g/dL).

Conclusion: The performance of PCT assay for LUMIPULSE G1200 and G600II was satisfactory. The measurement value was compatible for the other B•R•A•H•M•S PCT assay. On the other hand, the accuracy and reproducibility at especially low range is one of the best in current PCT assay. The performance of Lumipulse *G* B•R•A•H•M•S PCT is considered as useful for the routine analysis of PCT.

B-085

Evaluation of the Architect Syphilis assay after reformulation

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

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

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

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

B-086

Zika virus in Brazil: a prevalence study

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Background: Zika virus (ZIKV) is an RNA virus of family *Flaviviridae* and genus *Flavivirus* transmitted by mosquitoes of genus *Aedes*, including specie *Aedesaegypti*. It was initially isolated in 1947 in the Zika forest in Uganda, and nonspecific clinical features of Zika fever (FZ) can be confused with most other arboviruses particularly dengue and chikungunya virus infection. The first ever case of ZIKV disease in Brazil was reported in May 2015 and since then the virus has rapidly spread within Brazil and across 22 other countries. Currently, 534 out of 72,062 Zika suspected cases reported in Brazil were confirmed. The outbreaks suggests that FZ is an emerging disease and its might be associated with the increasing numbers of congenital microcephaly cases reported in the country. Therefore, there is an urgent need to detect and study the prevalence of Zika virus in Brazil and regions in order to contribute to the implementation of public health policies in Brazil. **Objective**: To report the prevalence of Zika virus infection in Brazil and federative units during period of December 2015 to February 2016.**Methods**: The detection of ZIKV was performed by RT-qPCR with specific primers and TaqMan fluorescent probe technology. The inclusion criterion was the detection of viral RNA in patients. **Results**: A total of 585 patients were analyzed, among these 137 (23.4%) from Midwest region, 145 (24.8%)

from Northeast region, 42 (7.2%) from North region, 230 (39.3%) from Southeast region, 31 (5.3%) from South region. Only one state (Acre) has not been studied. It was observed that the median age of patients was 35 years, andmost patients were female (73.8%).Among the samples evaluated 12.14% (71/585) were ZIKV positive. The Midwest region presented 19.7% (27/137) of positive cases, North region 19.5% (8/42), Southeast region 12.2% (28/230), Northeast region 4.8% (7/145) and South region 3.2% (1/31). The data showed that state of Bahia had 43% of positive results of Northeast region; Minas Gerais state had 75% of Southeast region, Goias state had 52% of Midwest region and Tocantins had 50% of North region. **Conclusion**: The results obtained by ZIKV specific molecular testshowed that Midwest region has the highest prevalence of zika virus infection followed by North region and Southeast region. However, the published data indicate that the Northeast is the most affected. Thisprevalence study of ZIKV infectionin different regions of Brazil may lead to a clearer indicationofvirus potential to spread and establish in the country. Further investigations are needed to improve our understanding the disease and its clinical effects such as possible connections between the virus and neurologic complications.

B-087

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

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Background: According to the World Health Organization more than 1 million sexually transmitted infections (STIs) are acquired every day worldwide. There are more than 30 different species of bacteria, viruses, and parasites that are sexually transmitted, and several of these agents can be transmitted during pregnancy and/or shortly after birth. STIs often involve more than one pathogen, difficulting the diagnosis and increasing the treatment cost, besides increase susceptibility to Human Immono deficiency Virus (HIV) infection. *Neisseria gonorrhoeae* and *Chlamydia trachomatis*are major causes of pelvic inflammatory disease and infertility in women. *Mycoplasma genitalium*is detected in urogenital tract and can cause urethritis in men and cervicitis, endometritis, and tubal factor infertility in woman. *Mycoplasma hominis* is associated with non-gonococcal urethritis, pelvic inflammatory disease, spontaneous abortions and infertility. It is often present concurrently with *Ureaplasma* species.*Ureaplasma urealyticum* is related with urogenital tract infections, abortions and infertility. **Objective:** To investigate the prevalence of *Mycoplasma hominis, Mycoplasma genitalium,Ureaplasma urealyticum, Neisseria gonorrhoeae* and *Chlamydia trachomatis* among patients tested for STIs. **Method:**This is a cross-sectional study of data from patients who were tested for STIs in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) in the period of 2010 to 2012. The inclusion criteria was the detection of DNA ofone or more microorganisms by using polymerase chain reaction. **Results:**From a total of 2,332 samples analyzed, 1,393 (59.7%) were positive for one or more microorganisms, and 939 (40.2%) were negative. 1,334 (57.2%) patientswere male and 998 (42.7%) female. *Mycoplasma hominis* was the most prevalent microorganism among both sexes, 509 (78.5%) men and 486 (71.2%) women. Overall, men had a higher positivity rate than woman, with 711 (51.0%) versus 682 (48.9%) for STIs. Among the positive samples, 1,160 (83.2%) were detected a single microorganism, and 233 (16.7%) were detected multiple microorganisms. 995 (71.4%) samples were positive for *Mycoplasma hominis*, 119 (8.5%) for *Chlamydia trachomatis*, 31 (2.2%) for *Ureaplasma urealyticum*, 13 (0.9%) for *Neisseria gonorrhoeae,* and 2 (0.1%) for *Mycoplasma genitalium*. 220 (15.7%) patients were coinfected by two microorganisms which *Mycoplasma hominis/Chlamydia trachomatis* were the most frequent, and 13 (0.9%) were coinfected with three microorganisms which *Mycoplasma hominis/Chlamydia trachomatis/Ureaplasma urealyticum*were the most frequent. **Conclusions:** The most prevalent STI was *Mycoplasma hominis,* followed by *Chlamydia trachomatis, Ureaplasma urealyticum, Neisseria gonorrhoeae* and *Mycoplasma genitalium*. The most prevalent coinfection was *Mycoplasma hominis/Chlamydia trachomatis*, which is consistent with the literature, and also *Mycoplasma hominis/Chlamydia trachomatis/Ureaplasma urealyticum*. It was not found data in the literature describing coinfection with three or more microorganisms. Further studies are necessary to investigate the prevalence of infections with multiple STIs, once this information can help in prevention, behavior modification counseling, partner notification, and early treatment continue to be the mainstays in preventing the spread of STIs.

B-088

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

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

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

Sensitivity

HIV Ag/Ab Combo

Antigen Sensitivity: The analytical HIV-1 p24 antigen sensitivity was determined by evaluation of the WHO International Standard HIV-1 P24 Antigen NIBSC (code: 90/636) and the BIO-RAD HIV-1 Antigen Standard (LN 72217).

Antibody Sensitivity: Clinical sensitivity was evaluated on selected HIV-1 (n = 430 including 43 HIV-1 gO specimens) and HIV-2 (n = 115), positive specimens. Seroconversion sensitivity was assessed on sequential specimens from 37 seroconverting donors.

Syphilis

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

Specificity

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

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

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

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

B-089

Concordance for HIV Combo assay between Chemiluminescence and Eletrochemiluminescence platforms

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Objective: The HIV diagnostic is ever a challenge to the laboratories, differences between methodologies, specificity and sensibility of the tests and the false reactive samples can be verified in the routine. This study aims to compare concordance of HIV combo assays between the platforms Cobas E-170 (Roche) and ADVIA Centaur XP (Siemens Healthcare Diagnostics) in a large laboratory in Cascavel, Brazil.

Materials and Methods: 2602 samples were selected from the laboratory routine, with concentrations within assay linearity range for each assay. Samples were tested

in ADVIA Centaur XP and Cobas E-170, which uses the chemiluminescence and electrochemiluminescence methods, respectively. The correlation was performed based in the first result without a duplicate repetition in both methodologies.

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

Total Samples	*2602*	
Concordant Results	**2531**	**97.27%**
Nonreactive Samples Agreement	2303	88.51%
Reactive Samples Agreement	228	8.76%
Discordant Results	**71**	**2.73%**
Reactive Siemens x Nonreactive Roche	41	1.58%
Nonreactive Siemens x Reactive Roche	28	1.08%
Reactive Siemens x Inconclusive Roche	1	0.04%
Nonreactive Siemens x Inconclusive Roche	1	0.04%

Table 1: Results of comparative.

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

B-090

Concordance for CMV assay between chemiluminescence and eletrochemiluminescence platforms

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

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

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

Total Samples	*CMV IgM*	*641*	*CMV IgG*	*578*
Concordant Results	**402**	**62.71%**	**571**	**98.79%**
Nonreactive Samples Agreement	339	52.89%	57	9.86%
Reactive Samples Agreement	50	7.80%	514	88.93%
Inconclusive Samples Agreement	13	2.03%	-	-
Discordant Results	**239**	**37.29%**	**7**	**1.21%**
Nonreactive Siemens x Reactive Roche	71	11.08%	7	1.21%
Nonreactive Siemens x Inconclusive Roche	57	8.89%	-	-
Reactive Siemens x Inconclusive Roche	13	2.03%	-	-
Reactive Siemens x Negative Roche	56	8.74%	-	-
Inconclusive Siemens x Negative Roche	32	4.99%	-	-
Inconclusive Siemens x Reactive Roche	10	1.56%	-	-

Table 1: Total Samples Results Classification

IgM discordant results were evaluated analyzing the Abbott IgM results as well as Avidity IgG results. When a patient have IgM variable between the methodologies and a high avidity, the IgM found were considered as a residual. Thus, of the 239 (37.29%) discordant results 21 (3.28%) are considered result resultant of residual for Roche test, 57 (8.90%) for the Siemens test and 9 (1.40%) for both.

Conclusion: The results showed a lower correlation for CMV IgM (62.71%) comparing with the CMV IgG assay (98.79%). Part of the differences in the results of CMV IgM is due the IgM residual results. The percentage of IgM residual could be observed in both assays analyzed. Those differences between platforms Siemens and Roche can be clarified in routine using a third assay and also the Avidity IgG Assay.

B-091

Concordance for HVA Total and IgM assay between chemiluminescence and eletrochemiluminescence platforms

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

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

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

Total Samples	**HAV Total**	**n= 279**	**HAV IgM**	**N=408**
Concordant Results	268	96.06%	405	99.26%
Nonreactive Samples Agreement	73	26.16%	341	83.58%
Reactive Samples Agreement	195	68.89%	64	15.69%

Table 1: Comparative results.

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

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

B-092

Concordance for HBV markers assay between chemiluminescence and eletrochemiluminescence platforms

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

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

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

Assay (Total Samples)		HBsAg (2473)	Anti-HBs (2258)	HBc Total (517)	HBc IgM (432	Anti-Hbe (441)	HBeAg (405)
Concordant Samples% (n)		97.9 (2420)	96.1 (2170)	97.5 (504)	98.1 (424)	94.3 (416)	99.0 (401)
Discordant Samples% (n)	NR Siemens x R Roche	0.9 (21)	1.3 (30)	2.5 (13)	_	0.5 (2)	_
	R Siemens x NR Roche	0.6 (15)	2.6 (58)	_	0.9 (4)	3.6 (16)	1.0 (4)
	R Siemens x INC Roche	0.3 (9)	_	_	_	_	_
	NR Siemens x INC Roche	0.3 (8)	_	_	_	_	_
	INC Siemens x NR Roche	_	_	_	0.2(1)	1.1 (5)	_
	INC Siemens xR Roche	_	_	_	_	0.5 (2)	_
Precision Results	Within RunPrecision	1.9%	2.0%	3.8%	4.8%	1.9%	3.4%
	Between RunPrecision	7.4%	3.1%	5.4%	6.5%	1.8%	8.7%

Table 1: Comparative and precision results.

Conclusion: The results showed a good correlation between the HBV markers assays analyzed, considering only the first result for each assay (initially reactive): HBsAg 97,86%, anti-HBs 96,10%, anti-HBc Total 97,49%, anti-HBc IgM 98,15%, anti-HBe 94,33% and HBeAg 99,01%. The differences in the results of HBV between platforms Siemens and Roche can be clarified by duplicate repetition and also third assay with similar methodology.

B-093

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

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

B-094

Concordance for Rubella IgM and IgG assay between chemiluminescence and eletrochemiluminescence platforms

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

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

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

Total Samples	Rubella IgM	497	Rubella IgG	471
Concordant Results	402	80.56%	411	87.26%
Nonreactive Samples Agreement	373	74.75%	32	6.79%
Reactive Samples Agreement	26	5.21%	378	80.25%
Inconclusive Samples Agreement	3	0.60%	1	0.21%
Discordant Results	95	19.04%	60	12.74%
Nonreactive Siemens x Reactive Roche	60	12.02%	3	0.64%
Nonreactive Siemens x Inconclusive Roche	24	4.81%	3	0.64%
Reactive Siemens x Inconclusive Roche	9	1.80%	19	4.03%
Reactive Siemens x Nonrective Roche	-	-	18	3.82%
Inconclusive Siemens x Reactive Roche	2	0.40%	3	0.64%
Inconclusive Siemens x Nonreactive Roche	-	-	14	2.97%

Table 1: Total Samples Results Classification

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

B-096

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

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

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

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

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

B-097

Pleural fluid lactate for diagnosis and management of parapneumonic pleural effusion

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Background: Parapneumonic pleural effusions (PPE) are exudates associated to pneumonia, lung abscess or bronchiectasis, they are called empyemas when containing pus. Approximately 40% of patients hospitalized with pneumonia have pleural effusions. Morbidity and mortality are higher in patients with pneumonia and pleural effusion than in those without pleural effusion. In the management of PPE, the relevant challenge is to discriminate between uncomplicated (UPPE) and complicated PPE (CPPE) for identify which PPE require chest tube drainage (CPPE and empyemas). Pleural fluid lactate levels may be useful for diagnosis and management of PPE; however, there are very few published studies on the quantification of lactate in pleural fluid. New studies assessing the diagnostic value of pleural fluid lactate are necessary. The aim of this study was to measure the accuracy of pleural fluid lactate concentration for diagnosis of PPE and to discriminate between UPPE and CPPE. **Methods:** We studied pleural fluids obtained by thoracocentesis in patients with pleural effusion. After to centrifugation of sample in sterile tube at 4000 rpm for 5 minutes, lactate was measured in the supernatant of pleural fluid using automated analyzers Dimension EXL (Siemens Diagnostics®). The reference interval of plasma lactate with this method is < 2.1 mmol/L. Patients were classified into two groups according to the etiology of pleural effusion: PPE and NOT PPE. The PPE patients were considered: a) Patients with pleural effusion and pneumonia, abscess, or bronchiectasis diagnosed by radiography, computed tomography or magnetic resonance imaging; or b) Patients with Gram stain or positive culture of pleural fluid. Statistical analysis was determined using receiver operating characteristic (ROC) techniques, analysing the area under the ROC curve (AUC) by the software MEDCALC®. **Results:** We studied 173 patients with ages between 1 to 96 years (median = 64 years), 83 women and 90 men. Thirty patients were PPE (10 UPPE and 20 CPPE) and 143 were NOT PPE (37 transudates, 88 malignant, 5 tuberculosos and 13 other etiologies). The AUC value was 0.831 ($p<0.0001$) and the optimal cut-off value was 5.6 mmol/L exhibiting 70% sensitivity and 90.9% specificity for diagnosis of PPE. Also, pleural fluid lactate could be use to discriminate between UPPE and CPPE, the AUC value was 0.740 ($p=0.0089$) and the optimal cutoff value was 10.2 mmol/L, exhibited 45% sensitivity and 90% specificity. **Conclusions:** Pleural fluid lactate has a high accuracy for diagnosis and management of PPE. Pleural fluid lactate > 5.6 mmol/L may be included among the biochemical parameters used to define PPE, and pleural fluid lactate > 10.2 mmol/L to identify which PPE require chest tube drainage.

B-098

Total bile acid levels in hepatitis C RNA positive patients with liver fibrosis

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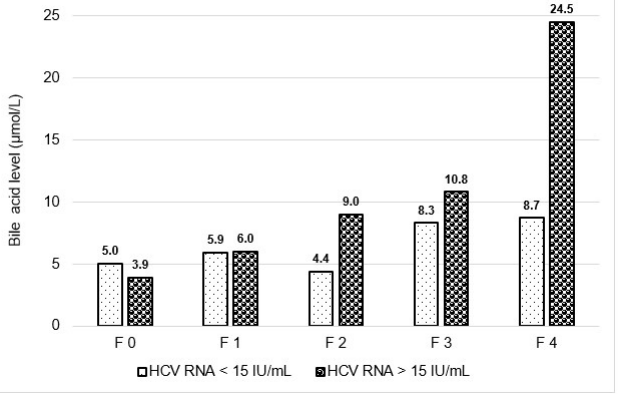
Background: Bile acids (BA) are liver-specific products of cholesterol metabolism, and their measurement in blood is used as test of hepatobiliary dysfunction. Furthermore, BA have been associated with increased viral replication in hepatitis C virus (HCV) infected cells. BA levels were also shown to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

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

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

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

Figure 1. Mean Plasma Bile Acid Levels and HCV Viral Load



B-099

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

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Kawasaki disease (KD) is the most common systemic vasculitis syndrome that primarily affects infants and young children. KD is in most cases a self-limited illness resolving within few weeks after fever onset. It preferentially affects small and medium-sized arteries, particularly the coronary arteries, and in 15 to 25% of patients, it will result in coronary aneurysms or dilatation. The natural history of coronary artery lesions in KD furnish evidences for a continuous active pathological process after the decline of acute symptoms. According to histological studies, even apparently normal coronary artery with no history of dilated lesions could show some degree of intimal thickening several years after the disease onset. Consequently, effective markers of inflammation and autoimmunity are needed for an effective follow-up of KD children both during the acute and subacute phase as during the following years. Among several inflammation markers available, Calprotectin (CP), a calcium- and zinc-binding protein of the S100/calgranulin family has been successfully used as a marker in several inflammatory and autoimmune diseases. The objective of the present study is to evaluate the use of Calprotectin (CP) as a serological marker in the assessment of inflammation in patients during the different stages of KD. Thirteen patients (2.5 years ± 1.2) were selected from January 2013 to December 2014, among patients referred to the Brasilia Children's Hospital Jose Alencar (Federal District, Brazil) for KD diagnostic confirmation and follow-up. Serum samples were collected following standard H3-A6 of Clinical and Laboratory Standards Institute (CLSI) and stored at -80°C until analysis. Calprotectin was assayed by ELISA method using a commercial kit according to the manufacturer's instructions. For analysis, the samples were stratified into three groups according to the stage of KD (acute, subacute and chronic) and calprotectin levels were detected at three different times: during the first 10 days of disease (acute phase, n = 3), between 30 and 60 days (subacute phase, n = 4) and ≥150 days after diagnosis (chronic phase, n = 6). The acute phase sample results (up to 10 days of diagnosis) disclosed levels of CP of 231.2 ± 23.3 ng/mL. Levels of 68.43 ± 16.6 ng/mL were detected during the subacute phase, and levels of 113.6 ± 55.6 ng/mL during the chronic phase. Our preliminary data

indicate that high levels of CP can still be detected during the chronic phase of KD suggesting that an inflammatory process continues active. Further study on the presence sustained inflammatory process during KD evolution and on the use of CP as an inflammatory marker are needed and should be performed.

B-100

Robust and reliable Screening of HPV Subtypes by Mass Spectrometry

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

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

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

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

Conclusion: We conclude that the MassARRAY HPV panel represents a highly specific, sensitive and reliable method for the detection of HPV subtypes in FFPE samples and Pap smears in a high throughput setting.

The MassARRAY® System is For Research Use Only. Not for use in diagnostic procedures

B-101

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

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The goal of treatment in patients with chronic hepatitis C virus infection is to eradicate hepatitis C virus (HCV) RNA, which is predicted by the attainment of a sustained virologic response (SVR). A SVR is associated with a 99 percent chance of being HCV RNA negative during long-term follow-up. Treatment with pegylated interferon 2a or 2b combined with Ribavirin (RBV) has been considered the standard of care (SOC) for HCV treatment. Approximately 50% of patients with HCV exhibit Mixed Cryoglobulineamia which is a bengin but prelymphomatous condition. **Aim** of this work was to evaluate the clinical utility of interferon Lambda-3 polymorphism rs12979860 and its association with -871 C/T BAFF polymorphisms as predictors of viral clearance and treatment response as well as MC occurrence among HCV treated patients. **Patients**: Analytical cohort study conducted on 100 HCV patients. They received pegylated interferon and ribavirin as treatment of choice. All the patients have signed an informed consent. **Methods**: HCV quantitation by COBAS AmpliPrep ,TaqMan PCR,Genotyping of HCV using the Abbott Real-time HCV Genotype II . Levels of BAFF mRNA were determined by real-time quantitative PCR. **Results:** Males were more non-responder than females. The level of viremia showed that a total of 68 patients were responders; 23.5% with very low level of viremia (< 200,000 U/ml) with the rest of responders had viremia level < 600,000 to 800,000 U/ml . All responders achieved RVR after 4 weeks and maintained e-RVR after 12 weeks and predict more than 90% SVR. IL-28B polymorphysim rs12979860 showed 68 patients who were responders; 22 (32.4%) of them had IL-28B genotype CC, 37 patients of them (54.4%) had CT type, and 9 patients (13.2%) had TT genotype. The other 32 patients who were non responders showed increasing in CT genotype in 81.2% and 8.8% for TT genotypes. There was statistically significant difference between IL-28B polymorphism C allele in responders group for treatment (MCP=0.002) (P≤0.05).Regarding the BAFF -871 C/Tgenotype the TT genotype was significantly increased in HCV patients whether with MC or without compared with the control group (P=0.036) . Among responders with MC 52 patients were positive for TT with 16 patients with CT genotype which was proved to be statistically significant with P <0.001 when compared with patients without MC . 6 Patients showed CT polymorphism, while those without MC; Baff -871 TTgenotype for CC was positive among 58 patients, 4 patients with CT polymorphism which proved to be statistically significant with P <0.001. **Conclusion**: in the present study, we demonstrated that CC genotypes of rs12979860 significantly determined SVR in patients with HCV mainly genotype 4.Carrying at least one copy of the C allele increased sensitivity to PEG-IFN/Ribavirin therapy, which was 3 times that of rs12979860-negative hosts. The prevalence of the BAFF -871TT genotype was significantly associated with the formation of Mixed Cryoglobulineamia and could be used as a marker for follow up.

Key Words : HCV , PCR , BAFF (B Cell Activating Factor)

B-102

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

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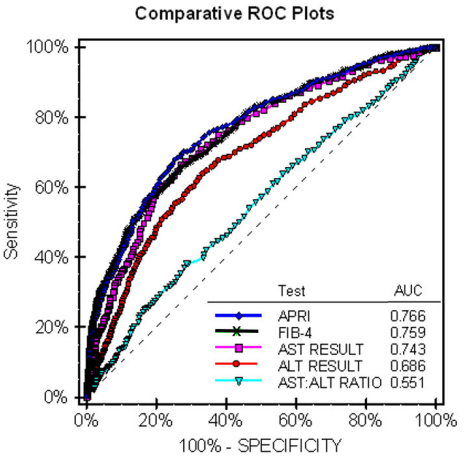
Background: With the recent availability of direct-acting antiviral drugs for hepatitis C virus (HCV) capable of inducing sustained virologic responses, efficient tools are needed to rapidly identify higher stage HCV-infected patients to prioritize them for early treatment. Fibroscan (transient elastography)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

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

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

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



B-103

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

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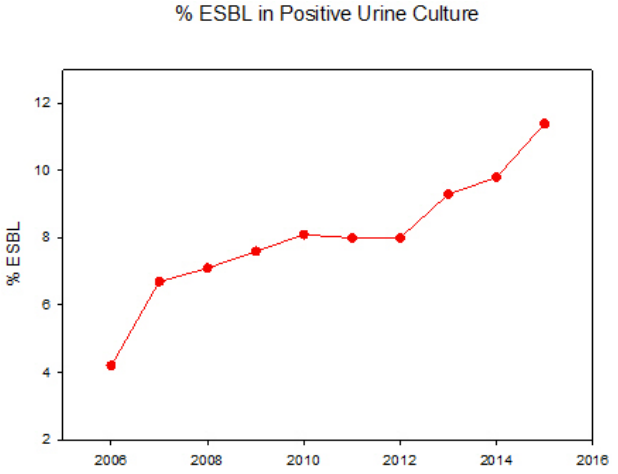
Background: Urinary tract infection (UTI) is one of the most common infections in the geriatric population; it is considered the second cause of community-acquired infection and nosocomial infection. Most of the UTI in long-term care facilities are caused by gram negative bacteria; however, Extended-Spectrum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

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

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



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

B-104

cerebro spinal fluid adenosine deaminase levels in tuberculous and pyogenic meningitis

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Background:

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

Methods:

60 clinically symptomatic cases of meningitis admitted between 16 and 50 years of age in medical wards of King George Hospital, Visakhapatnam, India between January 1999 and November 1999 were enrolled the present study. Out of these 60 cases there was 23 cases with tuberculous meningitis, 9 cases were diagnosed as pyogenic meningitis and remaining with other causes which were not included in study. There were 12 normal adults were also in this whose diagnosis was not meningitis. Lumbar puncture was done on the day1, day 8 and day 30 for tuberculosis patients, day1 and day 8 for pyogenic meningitis patients and was only once to the controls. CSF was collected and examined for levels of Adenosine Deaminase by authentic method. Anti tuberculous treatment was started for tuberculous meningitis patients and anti bacterial treatment was started for pyogenic meningitis patients.

Results: The levels of adenosine deaminase significantly high in both pyogenic and tuberculosis patients when compared with controls p-value < 0.05 (3.86+/-0.15). The levels of adenosine deaminase was significantly decreased on day 8 after treatment p-Value < 0.05(13.04+/-1.74 on day 1 and 9.4 +/- 1.2 in day 8.). In tuberculous meningitis it was the levels of adenosine deaminase levels were significantly elevated on day 8 after treatment(p-value < 0.05) and significantly reduced on day 30 of the treatment (p-value < 0.05) (15.08+/- 3.15 on day 1 ,17.99+/-4.81 on day8 and 9.4+/-1.9 on day 30) .

Conclusion:

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

B-105

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

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

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

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

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

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

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B-106

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

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

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

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

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

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

relevance for adult patients, it may be of particular interest in pregnant women, possibly reflecting viral replication in fetal tissues.

Lanciotti et al. Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007. Emerging Infectious Diseases Vol. 14, No. 8, 2008.

B-109

Detection of NDM-1 and CTX-M-type producing *Enterobacteriaceae* strains in Brazil

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Background: *New Delhi Metallo β-Lactamase* (NDM) has become one of the main globally described carbapenemases. The first report of NDM happened in 2009 in India. Four years later, in 2013, Brazilian researchers reported the first description of NDM in our country in a *Providencia rettgeri* samples. Since then, the emergence of this mechanism of resistance has been described in different species of Gram-negative samples in Brazil. The objective of this study was report the detection of NDM-1 producing *Enterobacteriaceae* clinical isolates recovered at Brazilian hospitals. **Methods:** In January 2015 and in January 2016 five carbapenem-resistant *Enterobacteriaceae* strains were isolated from five patients hospitalized in two tertiary Brazilian hospitals. These strains were isolated from Urine (n=2), catheter tip (n=1) and rectal swab (n=2). The bacterial identification was performed using mass spectrometry (Vitek-MS, bioMerieux) and the minimal inhibitory concentration (MIC) of antibiotics were determined using the Vitek 2 System (bioMerieux), as well as E-test method, according to CLSI guidelines. The screening test of carbapenemase was performed by phenotypic assay using commercially available disks containing carbapenems with and without EDTA (0.1 M), cloxacillin (75 mg/mL) or phenylboronic acid (40 mg/mL), as recommended by the Brazilian National Health Surveillance Agency (ANVISA). Then, the detection of the carbapenemase ($bla_{KPC,}$ bla_{NDM}, bla_{IMP}, bla_{VIM}, bla_{GES} and $bla_{OXA-48-like}$) genes and ESβL genes ($bla_{TEM,}$ bla_{SHV} and bla_{CTX-M}) was determined by PCR followed by Sanger sequencing analysis of the genes. Genetic relatedness of the strains was characterized by Pulsed-Field Gel Electrophoresis (PFGE) using the *Spe*I restriction endonuclease. **Results:** Among the five *Enterobacteriaceae* studied, three isolates were identified as *Providencia rettgeri* and two as *Klebsiella pneumoniae.* In all of them, high-level of resistance were detected to ceftazidime (MIC, >16 µg/mL), ceftriaxone (MIC, >4 µg/mL), cefepime (MIC, >16 µg/mL), ertapenem (MIC, >2 µg/mL), imipenem and meropenem (MIC, >4 µg/mL). One of *K. pneumoniae* strains was also resistant to amikacin (MIC, 32 µg/mL) and colistin (MIC, >16 µg/mL). All strains showed the diameter-zone difference >5 mm between the carbapenem/ EDTA and carbapenem disks, screened as possible metallo-β-lactamase (MβL) producers. The presence of bla_{NDM} and bla_{CTX-M} genes was detected in all strains. All *P. rettgeri* strains showed an identical PFGE pattern. The PFGE protocol to *K. pneumoniae* strains is running. **Conclusion:** An early detection by screening methods could be very helpful for surveillance and infection control measures. In addition to that, the findings of the identical PFGE pattern among *P. rettgeri* suggest the occurrence of a horizontal dissemination and an emergence of this important mechanism of resistance in Brazil.

B-110

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

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

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

B-112

Clinical Performance of Elecsys® Anti-HCV II in Subjects with Increased Risk of Hepatitis

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

Specimens (serum, sodium citrate plasma) were tested in four US sites using Elecsys® Anti-HCV reference assay. Final HCV interpretation was based on a testing algorithm for reference reactive samples: two comparator immunoassays, Abbott Architect Anti-HCV and ORTHO VITROS a-HCV, and HCV RNA determination (Roche AMPLICOR Hepatitis C assay) for comparator-indeterminate samples.

Positive /negative percent agreements with 95% confidence limits are listed below.

Cohort	Positive		Negative	
	n	Percent Agreement, CL	n	Percent Agreement, CL
Adult[A]	560	99.64% 98.72-99.96%	1683	98.81% 98.17-99.27%
Pediatric[B]	32	100.00% 89.11-100.00%	197	98.48% 95.61-99.68%
Pregnant[C]	63	98.41% 91.47-99.96%	207	99.52% 97.34-99.99%

[A]Agreements were comparable in these prospectively-collected asymptomatic/symptomatic cohorts and ranked risk subgroups. No discrepant results were observed in co-infected subjects: 43 acute/chronic hepatitis B, 3 acute hepatitis A.
[B]192 subjects were US prospectively-collected; 37 specimens were acquired ex-US retrospectively.
[C]205 subjects were US prospectively-collected; 65 specimens were acquired US retrospectively.

Twenty-nine Flaviviridae (West Nile Disease, Dengue Fever, Murray Valley Encephalitis, Kunjin Fever) and 289 other specificity specimens representing 28 diseases were concordant between both assays.

Seroconversion sensitivities in both assays were equivalent in ten commercial panels; Elecsys® Anti-HCV II converted earlier in six panels and later in one panel.

Both assays demonstrated equivalent genotype recognition in three genotype panels.

Imprecision (CLSI EP5-A2) was evaluated using three reagent lots and two operators in three US sites. Three replicates imprecision pools were tested in two runs/day for

five days. All %CVs for Repeatability and Reproducibility were <3.1% and <12.0%, respectively. The C5–C95 (CLSI EP12-A2) interval ranged from 0.97-1.06 COI.

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

B-114

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

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