Wednesday, August 2, 2017

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

Infectious Disease

B-049

biochemical Effects of Ethanolic Roots Extracts of *Uvaria charmae* on liver of Albino wistar rats infected with *Staphylococus aureus*

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Background: Man has utilized different medicinal plants, one of which is Uvaria charmae in the treatment of *Staphylococcus aureus* Infection.This work was carried out to determine the biochemical effects on liver enzymes aspartate aminotransferase ALT, alanine aminotransferase AST, alkaline phosphatase ALP of Swiss albino Wister rats infected with *Staphylococcus aureus* and treated with *Uvaria charmae*

Methods: Twenty five (25) adult Swiss albino Wister rats weighing 170g-220g were used. The control group (group 1) was administered feed and water, Group 2 was infected with *Staphylococcus aureus* only, treatment group (group 3) was infected with *Staphylococcus aureus* and treated with *Uvaria charmae* extract for 10 days, comparison group (group 4) was infected with S. aureus and treated with Vancomycin for 10days, Group 5 was administered with uvaria charmae extract only for 10 days

Results: In analysis, AST levels were elevated in group3 (170.25±12.764), group 4 (120.00±7.071), group5 (179.00±3.742) as compared with the control group (46.00±5.657). This was statistically significant (P=0.001). Alanine aminotransferase (ALT) levels were elevated in group3 (174.75±3.745), group4 (104.50±3.775), group5 (111.25±5.377) as compared with the control group (36.50±2.121) and was statistically significant (P = 0.001). Alkaline phosphatase (ALP) was elevated in group3 (303.75±5.315), group4 (263.50±39.854), group5 (325.25±8.139) as compared to the control group (155.50±9.192). This was statistically significant (P =0.001). The Tukey post hoc test shows in AST, there was statistical difference between group2 and control (P =0.001), group3 (P =0.0001) and group4 (P = 0.015). For ALT levels, there was statistical significant difference between group2 and control (P =0.000), group3 (P =0.000), group4 (P =0.006) and group5 (P = 0.001). For ALP levels, there was statistically significant difference between group2 and group5 (P =0.007), group5 and group4 (P =0.009)

Conclusion: Hyper production of this enzymes could constitute a threat to the life of cells that are dependent on a variety of the phosphate esters for vital life processes (Butterworth & Moss, 2002) . This pattern of increase of ALT, AST and ALP observed in this study are biochemical indicators of liver cytolysis suggesting that the extracts may have adverse effect on the liver especially on continuous usage. AST and ALT occupy a central position in amino acid metabolism of the tissues and thus alteration in their activity by excessive hyper production affect normal growth of the tissues (Pantosi et al., 1997).Thus suggesting that *Uvaria charmae* may be unsafe even though utilized in commercially sold herbal drug like Ruzu bitters.

B-050

Detection of biofilm in *Staphylococcus aureus* isolated from wound infection and its association with antimicrobial resistance

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Background: *Staphylococcus aureus* including methicillin-resistant *staphylococcus aureus* (MRSA) has the propensity to form biofilms, and cause significant mortality and morbidity in the patients with wounds. The aim of this study was to determine the prevalence of biofilm formation by *S. aureus* isolated from wound infection, and to evaluate its antimicrobial resistance pattern. **Methods:** A total of 43 clinical isolates of *S. aureus* were isolated from 100 pus samples using standard microbiological techniques. Biofilm formation in these isolates was detected by tissue culture plate method and tube adherence method. Antimicrobial susceptibility test was performed using the modified Kirby-Bauer disk diffusion method as per CLSI guidelines. MRSA was detected using the Cefoxitin disk test.

Results: Biofilm formation was observed in 30 (69.7%) and 28 (65.1%) isolates of *S. aureus* via TCP and TM methods, respectively. Biofilm producing *S. aureus* exhibited a higher incidence of antimicrobial resistance when compared with the biofilm non-producers ($p < 0.05$). Importantly, 86.7% of biofilm producing *S. aureus* were multidrug resistance (MDR), whereas all the biofilm non-producers were non-MDR ($p < 0.05$). Large proportions (43.3%) of biofilm producers were identified as MRSA; however, none of the biofilm non- producers were found to be MRSA ($p < 0.05$). **Conclusion:** This is the first study to determine the prevalence of biofilm forming *S. aureus,* including MRSA, isolated from wound infections in Nepal. The occurrence of antimicrobial resistance was higher in the biofilm producers than in the non-producers. Therefore, we recommend screening for the detection of biofilm formation and monitoring the antimicrobial resistance profiles of *S. aureus,* which may help in formulating effective antimicrobial strategies when dealing with wound infections.

B-051

Frequency of false positive IgM anti-hepatitis B core antibody results

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Background: IgM antibody to hepatitis B virus core antigen (IgM anti-HBc) is present in patients with acute hepatitis B virus (HBV) infection or with reactivation of disease in chronic carriers. Total anti-HBc (both IgM and IgG) antibodies are detected in patients with either chronic HBV infection, past infection with immunity, or acute infection. Furthermore, false positives are well documented and especially common in populations with low prevalence of HBV infection. However, the frequency of false positive IgM anti-HBc results is unknown. The objective of this study was to evaluate the rate of false positive IgM anti-HBc results in a large veteran population.

Methods: A retrospective observational study covering a 5 year period through October 2016 was conducted with information extracted from the Veterans Affairs corporate data warehouse. Data collected included all anti-HBc IgM results in which total anti-HBc was also tested within the previous 7 days. Positive IgM anti-HBc results were classified as true positives if total anti-HBc results were positive or as false positives if total anti-HBc results were negative.

Results: Results from 52,426 paired IgM and total anti-HBc tests among 44,377 patients from 110 facilities were analyzed. Within this group, the median number of paired tests per facility was 154.5 with 10th to 90th percentile range of 22.5 to 1105.5. Total anti-HBc was positive in 28,810 (55%). The median rate of positive IgM anti-HBc tests per facility was 1.2% with 10th to 90th percentile range of 0.0% to 4.1%. Overall, a total of 662 (1.3%) IgM anti-HBc results were positive among 551 patients of which 59 (8.2%) were classified as false positives (negative total anti-HBc) among 50 patients. False positive IgM anti-HBc results persisted from 3 to 330 days in 7 patients retested 2 to 3 times.

Conclusion: This observational study provides evidence that positive IgM anti-HBc measurements are sometimes erroneous. While it is possible that some discrepant results could have been due to false negative total anti-HBc measurements, this has been rarely reported. In contrast, false positive total anti-HBc results are well documented. Therefore, it is likely that this would also occur with IgM anti-HBc measurements. Other causes contributing to discordant results such as mislabeling or clerical errors could not be evaluated, but would only marginally impact false positive rates. False positive IgM anti-HBc results might also be due to greater method sensitivity than total anti-HBc measurements. However this explanation is also unlikely due the persistence of false positive IgM anti-HBc results observed upon retesting days to months later. In conclusion, these results suggest that positive IgM anti-HBc results should be cautiously interpreted unless reflex testing with total anti-HBc is performed for confirmation. Alternatively, when acute HBV infection is suspected, total anti-HBc could be tested first and if positive followed by IgM anti-HBc measurement. This latter testing algorithm might be preferred since a negative total anti-HBc result would help to simultaneously exclude the diagnosis of acute as well as past or chronic HBV infection.

B-052

Comparison of Two Assays for Measurement of Quantitative Hepatitis B Surface Antigen in Patients with Chronic Hepatitis B

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Background: Serum hepatitis B surface antigen (HBsAg) levels correlate with hepatitis B virus intrahepatic covalently closed circular (ccc) DNA and may be useful to predict treatment response. Currently two commercial platforms are available for HBsAg quantification in clinical practice: the Roche HBsAg QT and Architect HBsAg Quant. This study aimed to compare the results of both assays.

Methods: HBsAg levels were measured in 50 serum samples from chronic hepatitis B patients and subsequently analyzed for quantitative HBsAg levels using both assays.

Results: Correlation between results obtained from the Roche and Architect platforms was high ($r=0.989$, $p<0.05$). By Bland-Altman analysis, agreement between the two assays was close (mean difference between Roche and Architect: 0.001 log IU/mL; limit of agreement: -0.20 log IU/mL [95% CI: -0.25 - -0.15) to 0.21 log IU/mL (95% CI: 0.15-0.26). Two (4%) samples were not consistent (difference: 0.42 and 0.30 log IU/mL). We hypothesize that this discrepancy is caused by HBsAg mutants.

Conclusion: There is a high correlation and close agreement between quantitative HBsAg measurement using the Roche and Architect platforms. Clinical prediction rules derived from data from one platform can be applied to another. Both assays can be used interchangeably in clinical practice.

B-053

Transcriptional profile of macrophage infected with different violent Mycobacterium tuberculosis strains by RNA-seq

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Background: Tuberculosis caused by *Mycobacterium tuberculosis* (MTB) remains a significant public health problem, which leads to 2 billion individuals and approximately 10 million new infections every year throughout the world. With the continually emerged multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB, a comprehensive understanding of anti-TB immunity in the host is urgently needed. Transcriptomic analysis has potential to greatly increase our understanding of MTB infection, which reveals gene expression and the complex network of gene regulations at transcriptional level. However, the transcriptomic change of macrophage infected with different virulent MTB strains still poorly understood. Here, we studied the transcriptional profile of macrophage infected with H37Rv and H37Ra using RNA-Sequencing(RNA-seq).

Methods: THP-1 cells were primed with PMA for 24 hours. Three groups were set, H37Rv infection group, H37Ra infection group and control group. Each group was treated for 1 hour, 4 hours, 12 hours, 24 hours and 48 hours. Then cells were harvested for RNA isolation. The sequencing library were prepared and sequenced in half lane in flowcell of Illumina Hiseq 3000 high throughput sequencer. Differentially expressed genes were identified using the edgeR program.

Results: Top four pathways involved in differentially expressed genes between H37Rv and H37Ra groups for 4h infection were: lipid metabolism, small molecule biochemistry, vitamin and mineral metabolism and cell death and survival signaling. Top four pathways between H37Rv and H37Ra groups for 24h infection were: cell cycle, DNA replication, recombination, and repair, cell death and survival signaling. Only 1 pathway were common between 4h and 24h MTB infection.

Conclusion: Our study revealed the transcriptomic change of macrophage infected with different virulent MTB strains, which could help to gain a better understanding of the regulation system in MTB infection immunity.

B-054

Detection Of ESBL, MBL, & Ampc Producing Bacterial Isolates Causing Nosocomial Urinary Tract Infections With Special Reference To Biofilm Formation

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Background: Nosocomial infection is a global problem with multi facet outcomes. At present, the emergence of resistance to antimicrobial agents is a global public health problem which is well pronounced in developing countries. Microbial bio films pose great threat for patients requiring indwelling medical devices (IMDs) as it is difficult to remove them. It is, therefore, crucial to follow an appropriate method for the detection of bio films. **Methods:** The aim of this study was to determine the prevalence of bacteria causing nosocomial urinary tract infections & their antimicrobial resistance pattern with special reference to biofilm formation among the patients admitted at Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. The study was conducted during a period of February to July 2015. Six hundred thirty five clinical specimens; mid-stream & catheterized urine were subjected for bacterial culture and their antibiotic sensitivity test & test for the detection of biofilm formation was performed by following standard methods.

Results: Of the 635 specimens studied, 89 bacteria & two Candida spp. were isolated from 91specimens. The most common isolates (N=41, 46.1%) were *E coli*, (N=14, 15.7%) were *E faecalis*, (N=13, 14.6%) were *P aeruginosa*. In-vitro antibiotic susceptibility tests revealed that the Gram-negative bacilli were sensitive to Carbapenem, Polymyxin B and Colistin Sulphate while the Gram-positive cocci were sensitive to Tigecycline, Teicoplanin and Vancomycin. Tissue culture plate method detected 53 (59.5%) biofilm producers and 36 (40.5 %) biofilm non-producers. The most common Gram negative isolates producing biofilm (n=45) were, *Escherichia coli* (31 %) *Klebsiella pneumoniae* (13.3%), & *Pseudomonas aeruginosa* (29%), and biofilm producing Gram-positive organism (n = 8) was *Enterococcus faecalis* (57%). Among the Gram negative biofilm producers (N=45), 44 (97.7%) were found to be multidrug resistant. 23 (51.1%) were ESBL producers, 13 (28.8%) MBL producers, 4 (8.8%) AmpC producers & 1 (2.2%) ESBL & AmpC co-producer. Among biofilm non producers (N=30), 30 (100%) were found to be MDR, 18 (60%) ESBL producers, 4 (13.3%) MBL producers, 1 (3.3%) AmpC producer & 1 (3.3%) ESBL & AmpC co-producer. **Conclusion:** The findings showed that biofilm producers were more multidrug resistant and strong beta lactamases producers as compared to the non-producers leading to resistance to the commonly prescribed antimicrobial agents & difficulty in treatment of the infections. Necessary measures should therefore be taken to detect biofilm producers in the lab & to reduce the rate of biofilm formation in the clinical settings.

B-055

CD4+ T-lymphocytes counts in children with Human immunodeficiency virus infection at the Paediatric Department of Korle Bu Teaching Hospital in Ghana

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Background: The high cost of CD4 count estimation in resource-limited countries is a major challenge in initiating patients on highly active antiretroviral therapy (HAART). Since laboratory assessments of HIV-infected patients by flow cytometric methods are expensive and unavailable in resource-limited countries, total lymphocyte count by haematology cell counter is supposed to be a suitable surrogate marker to initiate and monitor course of the disease in these patients. The aim of this study was to evaluate the utility of total lymphocyte count as a surrogate marker for CD4 count in HIV-infected patients. **Methods**: In a prospective study 98 HIV-positive children between the ages of 5-16 years were evaluated for total and CD4 lymphocyte count. For correlation between CD4 count and total lymphocyte count, haemoglobin and haematocrit we defined cut-off values as 200 cell/µl, 1200 cell/µl, 12 gr/dl and 30%, respectively, and compared CD4 count with each parameter separately. Positive predictive value, negative predictive value, sensitivity and specificity of varying total lymphocyte count cutoffs were computed for CD4 count ≤ 200 cell/µl and ≤ 350 cell/µl. **Results**: Strong degree of correlation was noted between CD4 and total lymphocyte count (r: 0.590, $P < 0.001$). Mean and standard deviation of total lymphocyte count, haemoglobin and haematocrit in relation to CD4 count were

calculated which indicated significant correlation between these variables. Kappa coefficient for agreement was also calculated which showed fair correlation between CD4 200 cell/µl and total lymphocyte count 1200 cell/µl (0.35). **Conclusion**: This study reveals that despite low sensitivity and specificity of total lymphocyte count as a surrogate marker for CD4, total lymphocyte count is of great importance and benefit in resource-limited settings.

B-056

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

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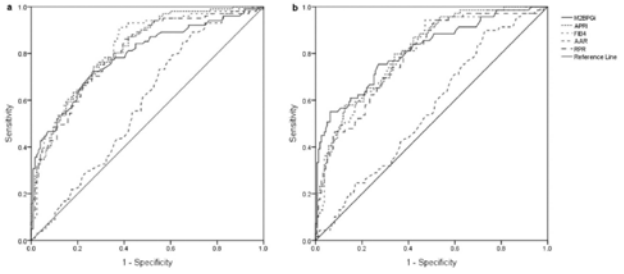
Background: Liver fibrosis is the common consequence of chronic hepatitis B virus infection, leading to the formation of cirrhosis. Accurate assessment of liver fibrosis is specifically essential for the management of patients with liver cirrhosis accompanied by distortion of hepatic vasculature. Currently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

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

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



B-057

Evaluation of the Bio-Rad Geenius HIV 1/2 Confirmation Assay as an Alternative to Western Blot for visa screening protocol in UAE

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Background: For several decades, western blot (WB)has been the gold standard as a confirmation assay after reactive enzyme immune assay(EIA) for human immunodeficiency virus(HIV) antibody. In April 2014, updated recommendations for diagnosis of HIV infection by the US Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) suggest a new diagnostic algorithm.Aim of work: to evaluated the Geenius HIV1/2 as a rapid, simple, and reliable alternative to WB for visa screening program in UAE. **Methods:** This study was conducted at National Reference Laboratory, Abu Dhabi ,UAE. Forty-nine samples were selected based on the HIV infection status of the patients, defined as positive (confirmed HIV-positive by WB) or negative (screening test negative). All serum specimens were initially determined by the 4th generation HIV antigen/antibody assay (Elecsys® HIV combi PT 4th Gen,Roche), and when reactive, we performed duplicated tests. All serum specimens were tested by the Geenius HIV1/2. WB was performed for all reactive samples. According to the new recommendation, sera with reactive HIV antigen/antibody assay but nonreactive or indeterminate Geenius HIV1/2 results should be further tested with HIV-1 nucleic acid amplification test (NAT) **Results:** In our study we consider WB as a gold standard method for evaluating the Geenius HIV 1 /2 differentiation assay. As according to the new algorithm HIV-1 NAT is included as a next step in differentiation assay. Overall sensitivity and specificity of the Geenius HIV 1/2 based on WB were (100% and 97.2%) respectively. Our results showed a high agreement between WB and Geenius assay for both HIV-negative individuals and HIV- positive infection. **Conclusion:** In view of previous results we recommend the use of genius HIV1/2 assay instead of WB for visa screening program in UAE.HIV-1 NAT should be performed in negative or indeterminate specimens by the Geenius HIV1/2 to detect acute HIV infection as recommended in new HIV testing algorithms.

B-058

Quantitative Detection on Serological Markers for Hepatitis B Virus Infection by Chemiluminescence Immunoassay

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Abstract

Purpose: Hepatitis B virus (HBV) infection has been a worldwide health problem nowadays and quantitative detection of its serological markers allows identifying or accessing HBV infection phases, monitoring antiviral therapy and may help elucidating the natural course of HBV infection. Thus, the main aim of the present study was to evaluate quantitative performance of Hybiome CLIA detection reagents for HBV serological markers on HYBIOME AE-240 platform.

Methods: Quantitative CLIA reagents for HBV serological markers manufactured by HYBIOME were evaluated on HYBIOME AE-240. CLIA reagents for HBsAg and anti-HBs of Company A on ARCHITECT i2000sr and time-resolved fluorescence immunoassay (TRFIA) reagents for HBeAg, anti-HBe and anti-HBc produced by Company F on TALENT-II were used as the reference reagents respectively. Comparison experiment about each marker quantitative detection reagent was performed in three different hospitals. Specimens (1245, 1230, 1087, 1112 and 1112 for HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc) from different donors were collected. The comparison study was approved by Ethic Committee of each hospital and was compliance with Guidance of *in vitro* Diagnostic Reagent for Clinical Study (China).

Results: Measured values of HYBIOME reagents for HBV serological marker detection had good linear correlation with the theoretical values in the linearity range of HYBIOME HBV reagents ((0.1~150)IU/mL for HBsAg, (7~500)mIU/mL for Anti-HBs, (0.5~8)IU/mL for Anti-HBc, (0.5~50)PEIU/mL for HBeAg, (0.25~4) PEIU/mL for Anti-HBe), and the linear correlation coefficients were all greater than 0.9900(r>0.9900). Positive, negative and total coincidence rates of Hybiome assay kits with their respective reference reagents were really high in clinic (99.33%, 99.37% and 99.36% for HBsAg; 98.57%, 96.28% and 97.97% for anti-HBs; 98.67%, 98.28% and 98.57% for anti-HBc; 100.00%, 100.00% and 100.00% for HBeAg; 98.85%, 98.77% and 98.80% for anti-HBe). In addition, clinical test results of HYBIOME reagents and the reference reagents showed good correlation, correlation coefficient r was 0.9917, 0.9912, 0.9869, 0.9910 and 0.9906 for serum HBsAg, Anti-HBs, HBeAg, Anti-HBe and Anti-HBc detection.

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

B-059

Study on resazurin based antimicrobial susceptibility test of *Staphylococcus aureus* using real-time thermocycler

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Background: Antimicrobial susceptibility testing is very important when it is sought to select appropriate antibiotics to treat bacterial infections and to monitor the spread of bacterial resistance. Early initiation of antibiotic therapy is directly associated with survival. Therefore, rapid antimicrobial susceptibility testing improves patient outcomes and reduces costs; the therapeutic period is brief and invasive procedures minimized. Many existing antimicrobial susceptibility tests (ASTs) (disk diffusion and commercial instrument-based methods) require ≥ 16-18 h. More rapid, accurate methods are required. Here, we developed a new experimental resazurin-based antimicrobial susceptibility test (Resazurin-AST) using cell viability indicator resazurin; we compared our results to those afforded by broth microdilution testing.

Methods: The reference strain *Staphylococcus aureus* ATCC 29213 and 43 *S.aureus* isolates from various specimens were included. Oxacillin and clindamycin (commonly used to treat *S.aureus*) were the antibiotics of interest. We used 20 and 40 μL volumes of all of bacterial suspension, antibiotic solution, and resazurin solution to ensure acceptance of growth rate. All strains were grown in real-time thermocycler; fluorescence values were measured. Growth inhibition gradients were established and their reproducibilities were verified employing the reference strain. These gradients were used to determine antimicrobial susceptibilities, and the results were compared to those of the broth microdilution method and VITEK®2. The strains were divided into three groups by their minimal inhibitory antibiotic concentrations (MICs) upon broth microdilution testing, and the growth inhibition gradients were analyzed.

Results: Of 44 strains including the reference strain, 21 were susceptible to oxacillin and clindamycin, 20 were resistant to both antibiotics, and 3 were oxacillin-resistant but clindamycin-susceptible. The acceptabilities of the growth rates when the volumes used were 20 and 40 μL were 67.6% and 90.0%, respectively. The growth inhibition gradients revealed by fluorescence measurements tended to increase over time in susceptible strains, and to decrease or increase slightly in resistant strains. Upon repeated testing of the reference strain, the growth inhibition gradient remained identical. When the test volumes were 20 μL and 40 μL, oxacillin-susceptible and -resistant strains were distinguishable at 100 min (cycle 20) or 80 min (cycle 16). All clindamycin-susceptible and -resistant strains were distinguishable at 65 min (cycle 13). The growth inhibition gradient of strains divided by MICs showed differences among susceptible group with low MIC value, susceptible group with high MIC value and resistant group of oxacillin. However, no obvious distinctions were evident within clindamycin-susceptible subgroups.

Conclusion: The Resazurin-AST for *S. aureus* yielded data matching those of the broth microdilution test. The test results were obtained within 2 h, thus, suggesting the application of the Resazurin-AST in clinical settings.

B-060

Comparison of the Traditional and Reverse Syphilis Screening Algorithms in Medical Health Checkups

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Background: The syphilis diagnostic algorithms applied in different countries vary significantly according to the local syphilis epidemiology and other considerations, including the expected workload, need for automation in the laboratory and budget factors. This study compared the usefulness of the traditional and reverse syphilis diagnostic algorithms in health checkups.

Methods: In total, 1,000 blood samples were obtained from 908 men and 92 women during their regular health checkups. Traditional screening and reverse screening were applied to the same specimens using automatic rapid plasma regain (RPR) and *Treponema pallidum* latex agglutination (TPLA) tests, respectively. Second treponemal tests were performed using the chemiluminescent microparticle immunoassay (CMIA) in the reverse algorithm.

Results: Among the 1,000 samples tested, 68 cases (6.8%) were reactive in reverse screening (TPLA) compared to 11 cases (1.1%) in traditional screening (RPR). The corresponding κ value of the traditional algorithm compared with the reverse algorithm was 0.191 (95% confidence interval = 0.060–0.322), which indicates slight agreement between the traditional and reverse algorithms. The traditional algorithm missed 48 cases [TPLA(+)/RPR(-)/CMIA(+)]. The median cutoff index (COI) of TPLA was higher in CMIA-reactive cases than in CMIA-nonreactive cases (90.5 vs 12.5 U).

Conclusions: The reverse screening algorithm detected subjects with possible latent syphilis who could be given opportunities for evaluating syphilis infection in health checkups. The COI values of the initial TPLA test may be helpful in excluding false-positives in TPLA tests.

Table 1. Comparison of the traditional and the reverse algorithms

Reverse algorithms	Traditional algorithms		Total	Agreement (%)		Kappa value (95% CI)
	Positive	Negative				
Positive	6	48	54	11.1	(6/54)	0.191
Negative	-	946	946	100	(946/946)	(0.060–0.322)
Total	6	994	1,000	95.2	(952/1,000)	

Abbreviation: CI, confidence interval.

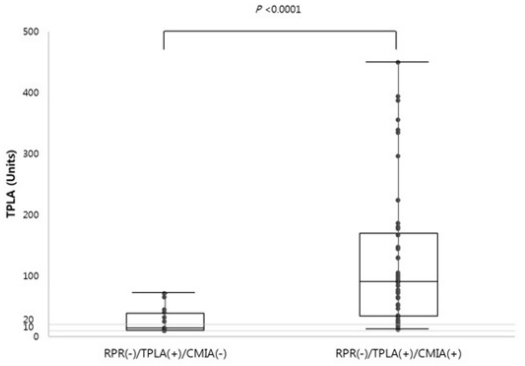

Fig 1. Comparison of the quantitative results of TPLA between CMIA-reactive and CMIA-nonreactive cases in the reverse algorithm.
Abbreviations: TPLA, *Treponema pallidum* latex agglutination; RPR, rapid plasma regain; CMIA, chemiluminescent microparticle immunoassay

B-061

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

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OBJECTIVES: Noninvasive markers of liver fibrosis in patients with chronic hepatitis C are needed for predicting disease progression. We aimed to establish a non-invasive diagnostic model to predict liver fibrosis development in hepatitis C virus (HCV)-infected patients in China. **METHODS:** One hundred and seventy-five patients with chronic HCV infection without other potential risk factors who underwent liver biopsy and serological tests were enrolled; factors evaluated included age, sex, viral load, fibrosis stage, aspartate and alanine aminotransferase, alkaline phosphatase, γ-glutamyltransferase, total protein, albumin, fasting glucose, total, bilirubin, direct bilirubin, total bile acids, creatinine, total cholesterol, triglycerides, platelet count, prothrombin time, thrombin time, activated partial thromboplastin time, hyaluronidase, type III collagen N-telopeptide, laminin, type IV collagen, anti-HCV IgG. For the formulation of diagnostic model, univariate logistic regression analysis was performed on variables between patients in the training set. Significant variables from the univariate analysis ($P < 0.05$) were then subjected to multivariate logistic regression analysis to identify independent factors associated with fibrosis. The fibrosis index derived from the training set was then applied to the validation set to test the predictive power of the selected model. Clinical data were compared with those for other noninvasive models (AST-to-platelet ratio (APRI), AST to ALT ratio (AAR), age-to-platelet ratio (API), cirrhosis discriminate score (CDS), FIB-4, and Forn's index) for estimating liver fibrosis using receiver operating characteristic (ROC) analysis. ROC curves were constructed to measure the diagnostic capacity of each test. **RESULTS:** The multivariate logistic regression analysis identified alanine aminotransferase (ALT), total bile acids (TBA) and hyaluronidase (HA) as independent risk factors for fibrosis. The model of ATH is consist of 3 indicators, ALT, TBA and HA, ATH= ln (ALT) × ln (TBA)× ln (HA). ATH was independently

associated with liver fibrosis stage as determined by liver biopsy(r = 0.716, P <0. 01). The cutoff values of ATH for fibrosis stages ≥S2, ≥S3, and S4 were 3.71, 4.72, and 6.21, respectively. The area under the receiver operating characteristic curve values (AUROC) of ATH for significant fibrosis (≥S2), severe fibrosis (≥S3), and cirrhosis (S4), were 0.877, 0.897, and 0.841, respectively. The AUROC of ATH model was significantly higher than alone ALT, TBA, HA index in fibrosis stages of significant fibrosis, severe fibrosis, and cirrhosis, respectively (P<0.01). ATH values offered a superior AUROC curve for the diagnosis of significant fibrosis, severe fibrosis and cirrhosis compared with the APRI, CDS, API, AAR, FIB-4 and Forns Index. Compared with the other noninvasive models s and scoring systems, ATH was the most useful marker for differentiating between fibrosis stages. For predicting liver cirrhosis (S4), ATH model had the highest AUROC (AUCATH=0.841, P<0.001) and for predicting severe liver fibrosis (≥S3), ATH model had the highest AUROC (AUCATH=0.897, P<0.001) and for predicting significant liver fibrosis (≥S2), ATH model also had the highest AUROC (AUCATH=0.877, P<0.001). **CONCLUSION:** The ATH model may be a simple, reliable, and non-invasive method to evaluate liver fibrosis in HCV-infected patients in China without an unnecessary liver biopsy.

B-062

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

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

Methods: Peripheral blood mononuclear cells and sera were collected from 44 healthy individuals (M:F=23:21, age= 35.8±11.2) and 26 CKD patients (M:F=13:13, age=45.7±11.6). BKV specific IgG levels were measured using qualitative Human BK Virus IgG ELISA Kit (MyBioSource, USA). The IFN-γ ELISPOT assays were performed to measure BKV-specific T cells (spots per $3x10^5$ lymphocytes) following the stimulation with different BK virus antigens (Large T antigen (LT), Small T antigen(ST), VP1, VP2 and VP3 antigens).

Results: BKV-specific IgG was detected in 59.1% of healthy individuals and 57.7% of CKD patients (P=0.909). The CKD patients demonstrated significantly increased BKV-specific ELISPOT results compared to the healthy individuals (LT, P <0.001; ST, P=0.002; VP1, P=0.019; VP2, P=0.002; VP3, P=0.002) The positive BKV-ELISPOT results (the cut-off > 10 spots/$3x10^5$) were more frequent in CKD patients versus healthy controls (LT, 76.9% vs. 47.7%, P=0.017; ST, 73.1% vs. 29.5%, P<0.001; VP2, 69.2% vs. 25.0%, P<0.001; VP3, 73.1% vs. 31.8%, P<0.001). LT and VP1 antigens were the most immunogenic proteins showing significantly higher ELISPOT results in healthy individuals (p=0.007, 0.001, respectively). In addition, LT and VP1 antigens induced a wide range of ELISPOT results in CKD patients (0-833 and 0-717 spots/$3x10^5$, respectively). However, BKV-ELISPOT results were not different between individuals with BKV-IgG(+) and BKV-IgG (-) (P>0.05). In BKV-ELISPOT (+) patints, the responses to different BKV antigens were highly heterogeneous, and the positive ELISPOT reactions to all five BK antigens were more frequent in CKD patients compared to healthy controls (53.9% vs. 11.4%, P <0.001).

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

B-063

Evaluation of two commercial immunoassays for detecting IgG and IgM antibodies against Epstein-Barr virus (EBV)

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Background: Antibodies against the capsid antigen IgG (VCA IgG) typically appear at the time of the onset of clinical symptoms of acute infection, and remain positive for life, whereas IgM antibodies (VCA IgM) usually appear at the same time as VCA IgG and disappear within a few weeks, although they may persist for several months. About 90% of adults throughout the world have antibodies against EBV. It is normally possible to distinguish acute from past infection associating VCA IgM and VCA IgG with EBV nuclear antigen (EBNA)-1 IgG. The presence of VCA IgM and VCA IgG without EBNA-1 IgG indicates acute infection, whereas the presence of VCA IgG and EBNA-1 IgG without VCA IgM is typical of past infection. The primary objective of this pilot study was to correlate the performance of Abbott-Architect® VCA IgG and VCA IgM against the same profile on Siemens- Immulite® 2000 systems. **Methods:** A total of 42 unselected serum samples, stored at -20°C, sent for EBV routine serological testing were assayed with ARCHITECT i2000 platform (USA) and Immulite 2000 (UK), both commercially chemiluminescent microparticle immunoassay. Data were submitted to EP evaluator® program for method comparison and statistical analysis. **Results:** The overall analytical performance assays was acceptable: 93,3% (82,1 to 97,7%) for EBV VCA-G, positive agreement 97,2% and negative agreement 77,8%, Cohen's Kappa 78,3%. EBV VCA-M qualitative method comparison also presented an overall agreement of 90,5% (77,9 to 96,2%), positive agreement 86,7% and negative agreement of 92,6%, Cohen's Kappa 79,3%. No sample of EBV VCA-M was excluded from data and "no agreement" was found in four samples, being two positive discordant and two negative discordant. Two samples of EBV VCA-G were negative and one sample positive with discordant results. **Conclusion:** Although there is good agreement between analytical platforms Siemens/Immulite® and Abbott-Architect, different results may be obtained. An isolated result of VCA IgM or VCA IgG should be cautiously interpreted when using only two parameters. Commercially available EBV nuclear antigen (EBNA)-1 IgG should be associated to distinguish acute and past infections in immunocompetent patients and pregnant women with symptons similar to cytomegalovirus.

B-064

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

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Introduction

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

Methods

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

Results

A total of 123 patients were tested on the GI Panel. The organisms detected were norovirus (n=10), *Salmonella* (n=7), *Shigella*/EIEC (n=2), *Campylobacter* (n=2), sapovirus (n=3), rotavirus (n=1), Giardia lamblia (n=2), Vibrio cholera (n=1), Cyclospora cayetanensis (n=1), Adenovirus (n=1), Enteroaggregative *E. coli* (n=1), Enteropathogenic *E. coli* (n=6), and Enterotoxogenic *E. coli* (n=1). These patients were compared with 594 historical controls who were tested using conventional stool test methods. 30/123 (24.4%) of cases were positive for at least one organism other than *C. difficile* compared with 41/594 (6.9%) of control patients. Two or more non-*C. difficile* organisms (co-infections) were identified in 4.9% (n=6) of patients who were tested on the GI Panel but no co-infections were found in our control population. Patients tested on the GI Panel had an average of 2.6 other infectious stool tests whereas the control population averaged 3.02 additional stool tests (p < 0.05). The number of abdominal radiologic exams was also slightly lower in the GI Panel group (0.35 vs 0.39) along with the length of stay following specimen submission (3.17 vs. 3.43 days); however, these differences were not significant. The average time from collection to result was 4.6 hours for patients tested by the GI Panel, compared with 54.75 hours for stool culture in the control population. Average days on antibiotics were 1.59 for the GI Panel group compared to 2.12 days in the control group (p<0.05).

Discussion

The FilmArray GI Panel has the potential to improve patient care by rapidly identifying a broad range of pathogens, reducing the need for other diagnostic tests, reducing unnecessary use of antibiotics, and leading to a reduction in hospital length of stay.

B-065

Prevalence of the infection of high-risk human papilloma virus among women in urban Changchun, northeast China: a 38260 female cases survey

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Background: Persistent infection of high-risk human papilloma virus (HR-HPV) is associated with cervical cancer. HR-HPV testing as a screening tool for cervical cancer is considered to be one of the more sensitive methods of screening for cervical cancer.

Objective: To investigate the prevalence of the infection of high-risk human papilloma virus among women in urban Changchun China.

Methods: This retrospective study was conducted between May 2011 and May 2015 at the First Hospital of Jilin University in urban Changchun China. All the women involved in this study, including 35568 female outpatients and 2692 healthy women, attended routine gynecological examinations and detected for high-risk human papilloma virus (HR-HPV) with Hybrid Capture 2.

Results: The overall positive rate of HR-HPV testing is 22.17% (female outpatients) and 18.29% (routine gynecological examination women) in urban Changchun northeast China from May 2011 to May 2015. The survey reflected a decreasing trend in the positive rate of HR-HPV testing, even as the total number of the woman for HR-HPV testing and the HR-HPV positive cases increased. Positive rate of HR-HPV testing of female out-patients increased and decreased with the season, but the positive rate of HR-HPV testing of healthy women who were attending routine gynecological examinations did not change a lot. Positive rate of HR-HPV testing of female outpatients among different age groups had statistical difference with each other. ①The positive rate of HR-HPV testing in 0~20 age group (39.59%) is higher than other age groups (except 61~65 age group) which has statistical significance. ②The positive rate of HR-HPV testing in 21~25,56~60 and 61~65 age groups (28.11%, 28.28%, 30.29%, respectively) had no statistical difference with each other, but lower than 0~20 age group and higher than any other age groups from 21 to 55 years old with statistical significance.③The difference of the positive rate of HR-HPV testing among the six age groups from 21 to 55 years old has statistical significant.④The positive rate of women ≥66 years old has no statistical difference with other age groups (except 0~20 age group). Positive rate of HR-HPV testing of routine gynecological examination women had no statistical difference between different age groups. The most common age of female outpatients and healthy women tested for HR-HPV is 41~45 years (19.01% and 21.77%, respectively), followed by 36~40 years (16.96% and 19.24%, respectively).

Conclusions: In Changchun northeast China, the overall positive rate of HR-HPV testing (22.17% and 18.29%) rank above average. For female outpatients, the positive rate of HR-HPV testing is higher in winter (Dec. Jan. and Feb.). The peak of the positive rate of HR-HPV testing appeared in young women (≤25 years old) and older women (56~65 years old). For routine gynecological examination women, the positive rate of HR-HPV testing doesn't change with the season and had no statistical difference between different age groups. The most common age of women tested for HR-HPV is 41~45 years old, followed by 36~40 years old.

B-066

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

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

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

Results :

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

In the test of enzyme linked fluorescent immunoassay, the cut-off level in Relative fluorescence value(RFV)

between "Negative" and "Equivocal" and between "Equivocal" and "positive" is 0.13, 0.34. ROC curve showed that the cut-off of 0.04 was 0.892 in Area under the curve (AUC) with 82.5 in sensitivity, 88.9 in specificity.

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

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

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

B-067

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

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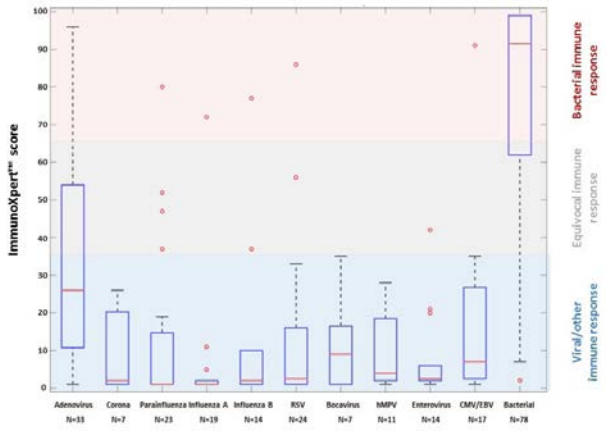
Background: A major challenge in effective management of febrile children is the clinical difficulty of distinguishing bacterial from viral infections. This uncertainty drives antibiotic misuse, hampering patient care and contributing to emergence of antibiotic resistance. ImmunoXpert™ is a novel assay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

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

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

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

Figure 1. Box plots of ImmunoXpert™ scores of patients presenting with different infection types
Blue boxes present first to third quartiles. Red line corresponds to group median. RSV - Respiratory syncytial virus; hMPV – human Metapneumovirus. Some patients presented with more than one viral strain



B-068

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

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

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

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

Validation: The LIAISON® XL Zika Capture IgM assay has been validated for precision, interference, and cross reactivity as well as clinical sensitivity and specificity. Intra and total assay imprecision are <9% and <14% respectively. The assay was evaluated for interference from endogenous substances including hemoglobin, serum albumin, bilirubin, triglycerides, cholesterol, HAMA and rheumatoid factor (RF). None of these compounds interfered in the assay. The assay was tested for cross reactivity to other related flaviviruses using samples that were positive for Dengue virus or West Nile Virus IgM, or from subjects who had been vaccinated for Yellow Fever. No cross reactivity was detected.

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

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

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

B-069

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

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Background

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

Objective

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

Methods

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

Results

Discrimination between sepsis (n=30, mortality=6.6%) and severe sepsis or septic shock (n=36, mortality=36.1%) revealed AUC values of 0.621, 0.627, 0.731, 0.740 and 0.781 for lactate, PCT, qSOFA, PSEP and the combination qSOFA+PSEP, respectively. 15 patients died during hospitalization. AUC values of mortality prediction were 0.715, 0.558, 0.734, 0.758 and 0.803 for lactate, PCT, qSOFA, PSEP and qSOFA+PSEP, respectively. qSOFA scores ≥2 should identify greater risk of death or prolonged ICU stay. Discrimination between qSOFA <2 and ≥2 revealed AUC values of 0.756, 0.669 and 0.606 for PSEP, lactate and PCT.

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

Conclusion

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

Detection rate of non-survivors and severe sepsis or septic shock					
Marker	Criterion	Non-survivors Detection rate	Non-survivors AUC	Severe Sepsis/ shock Detection rate	Severe Sepsis/ Shock AUC
PCT	2 µg/L	47%	0.558	20%	0.627
Lactate	2 nmol/L	75%	0.715	53%	0.621
PSEP	500 ng/L	93%	0.758	89%	0.740
qSOFA	2	67%	0.734	58%	0.731
qSOFA+PSEP	2/500 ng/L	93%	0.803	92%	0.781

B-070

Simultaneous detection of HIV- and hepatitis C- specific antibodies and hepatitis B surface antigen (HBsAg) by multiplex rapid diagnostic test

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Background: Multiplex rapid diagnostic test (RDT) for HIV, HCV and HBV may be advantageous in maximizing resources as these diseases share common risk factors and transmission modes. Exacto® HIV/HCV/HBsAg (Biosynex, Strasbourg, France) consists in manually performed, visually interpreted, immunochromatographic RDT simultaneously detecting in 15 minutes HIV- and HCV- specific antibodies (Ab) and HBV surface antigen (HBsAg) in serum, plasma and whole blood (venipuncture and fingerstick).

Methods: Hospital-based cross-sectional study was conducted on prospective panel of 750 sera from adult inpatients of hôpital Europèen Georges Pompidou, Paris, including 250 sera positive for HIV-specific Ab, 250 for HCV-specific Ab, 250 for HBsAg and 250 sera negative for HIV- and HCV- Ab and HBsAg, according to the results obtained by the reference Architect i2000SR (Abbott Diagnostic, Chicago, IL). Among HCV-seropositive sera, 187 were positive for HCV RNA (chronic infection), whereas 63 were negative (resolved infection), respectively. Serum samples were tested blindly by Exacto® HIV/HCV/HBsAg.

Results: Multiplex RDT showed very high sensitivity and specificity, and excellent concordance with Architect results. Lower sensitivity was observed only in individuals who had cleared their HCV infection. Mean lower limit of HBsAg detection was 5.1 IU/ml (PROBIT regression analysis).

Conclusion: Advantages of multiplex RDT for HIV, HCV and HBV include the requirement for less overall specimen volume, fewer finger-sticks if capillary whole blood is used, cost savings through lower cost per virus tested, improved patient flow with results for multiple viruses available at the same time, overall service delivery efficiencies with less time required per infected patient; and patient benefits from fewer visits and lower cost associated with each clinic attendance. In high risk population for HIV, HCV and HBV, which may be frequently combined, the screening of chronic HIV, HCV and HBV by multiplex Exacto® HIV/HCV/HBsAg RDT may clearly improve the "cascade of screening" and quite possibly linkage-to-care with reduced cost.

	Infectious status	Sensitivity (95% CI)	Specificity (95% CI)	Youden's J index	Cohen's κ coefficient
Anti-HIV Ab	Chronic	100% (99.9-100.0%)	100% (99.9-100.0%)	1.0	1.0
Anti-HCV Ab (HCV RNA+)	Chronic	100% (99.9-100.0%)	100% (99.9-100.0%)	1.0	1.0
Anti-HCV Ab (HCV RNA-)	Resolved	96.8% (92.3-100.0%)	100% (99.9-100.0%)	0.97	0.98
HBsAg	Chronic	100% (99.9-100.0%)	100% (99.9-100.0%)	1.0	1.0

B-071

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

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

B-072

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

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Background:

The simultaneous detection of Zika (ZIKV), chikungunya (CHIKV) and dengue (DENV) viruses provide a comprehensive diagnostic answer for patients. RT-qPCR is the most reliable method to detect and distinguish these three viruses. The assay is mostly performed on blood subproduts; the limitations are that the viruses RNA last for a few days and the absence of a high-throughput process (majority of RT-qPCR assays are manual/semi-automated). However, prolonged shedding of ZIKV and CHIKV in semen and urine has been reported and platforms for automated molecular tests have been released. Thus, the aim of the present study was to validate an automated and laboratory information system (LIS) integrated RT-qPCR workflow for simultaneous detection of ZIKV, CHIKV and DENV in EDTA-plasma, urine and seminal-plasma.

Methods:

The assay validation enrolled negative EDTA-plasma, urine and seminal-plasma pools/samples spiked with known amounts of each virus. The RT-qPCR workflow was provided by the Flow classic solution (Roche), which accepts different matrixes simultaneously. Nucleic acids were extracted from 500ul of each matrix (seminal-plasma was diluted 1:1 in PBS) by using viral NA universal LV 3.1 protocol. An in-vitro transcribed random RNA sequence was added into samples during the nucleic acids extraction and was co-amplified in all instances to function as a process control. Primers/probes were obtained from literature. ZIKV, CHIKV and DENV were assessed simultaneously, but in independent reaction wells. To determine the limits of detection (LODs), 1:1 dilutions of each virus were tested (from 1000 to 0.98 copies/mL), the LODs were calculated by probit regression analysis. The imprecisions near the clinical decision point (grey zones) were assessed by repeating the LOD experiment in triplicate during 5 days. The viral load ranges that did not allow certainty about the infection status were determined in each matrix. The accuracies were evaluated by a recovery strategy. The following numbers of samples were prepared for all tested specimens: ZIKV n=86 (21 positive), CHIKV n=85 (27 positive) and DENV n=84 (23 positive). Viral loads in positive samples ranged from $1x10^5$ to $5x10^2$ copies/mL in EDTA-plasma and urine and from $2x10^5$ to $1x10^3$ copies/mL in seminal-plasma. The agreements between the obtained and the expected results were evaluated.

Results:

The LODs in EDTA-plasma, urine and seminal-plasma, respectively, were 90.5 (95%CI 58-299), 51.6 (95%CI 40-74) and 136.3 (95%CI 111-183) copies/mL for ZIKV; 172 (95%CI 135-245), 112.6 (95%CI 92-150) and 551.8 (95%CI 451-730) copies/mL for CHIKV and 94.5 (95%CI 65-217), 38.8 (95%CI 31-53) and 1456 (95%CI 1205-2023) copies/mL for DENV. The grey zones in EDTA-plasma, urine and seminal-plasma, respectively, were 250-7.8, 62.5-3.9, 250-7.8 copies/mL for

ZIKV; 250-3.9, 250-7.8, 1000-31.3 copies/mL for CHIKV; and 125-7.8, 62.5-3.9, 2000-250 copies/mL for DENV. In the accuracy assay, the comparison between obtained and expected results revealed total agreement of 100% (95%CI 95-100%), positive agreement of 100% (95%CI 85-100%) and negative agreement of 100% (95%CI 94-100%) for all tested specimens. No cross-reaction was observed.

Conclusion:

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

B-073

Performance Evaluation of the ADVIA Centaur CMV IgM Assay

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Objective: Siemens Healthineers is currently developing a cytomegalovirus IgM (CMV IgM) assay to detect the presence of lgM antibodies to cytomegalovirus (CMV). Anti-CMV IgM antibodies are used as an aid in the diagnosis of recent or current CMV infection in individuals for which a CMV IgM test was ordered. The ADVIA Centaur® CMV IgM assay* is a chemiluminescent magnetic microparticle-based immunoassay that utilizes the NSP-DMAE molecule and runs on the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate the positive and negative agreement, precision, and cross-reactivity of a prototype automated CMV IgM assay tested on the ADVIA Centaur XP Immunoassay Systems.

Methods: The fully automated ADVIA Centaur CMV IgM assay is being developed as an indirect sandwich assay for the detection of CMV IgM antibodies in human serum and plasma for use on the ADVIA Centaur XP Immunoassay Systems. Positive agreement of the assay was evaluated with 104 samples that were positive by bioMerieux VIDAS and Siemens IMMULITE® 2000 CMV IgM assays. Negative agreement was determined by testing 300 pregnancy samples. The results were assessed based on Index values as reactive (index ≥1.0) and nonreactive (index <1.0). Samples from various disease conditions were tested for cross-reactivity. Precision was evaluated per CLSI EP5-A3 by testing six samples with Index values spanning the assay range in two runs per day for 10 days on the ADVIA Centaur XP system for a total of 40 replicates.

Results: Positive percent agreement of the ADVIA Centaur CMV IgM assay determined by testing CMV IgM-positive samples was ≥96%. Negative percent agreement determined by testing pregnancy samples was ≥98%. The assay was evaluated for potential cross-reactivity with other viral infections and disease-state specimens and no significant cross-reactivity. The assay demonstrated good precision with repeatability and within-run %CV of <4.0% and <6.0% respectively, for samples yielding Index values between 0.10 and 4.00.

Conclusions: The results of these studies demonstrate good performance of the prototype ADVIA Centaur CMV IgM assay on the ADVIA Centaur XP Immunoassay System.

*Under development. Not available for sale. The performance characteristics of this product have not been established.

B-074

Serological and molecular diagnosis of arboviruses in Brazil,2016

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

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

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

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

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

	MARKER		
	IgG	IgM	RNA
AGENT			
CHKV	33,762	33,651	382
DENV	18,873	21,825	150
ZKV	5,275	9,226	3,227

B-075

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

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

B-076

Performance Evaluation of the ADVIA Centaur *H. pylori* IgG Assay

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Background: *Helicobacter pylori* (*H. pylori*) is a gram-negative bacterium found in the human stomach that infects nearly 50% of the world's population. Most infected individuals are asymptomatic. However, *H. pylori* infection has been associated with gastritis, peptic ulcers, and gastric cancers. Diagnosis of *H. pylori* infection is mainly performed by a gastric biopsy or the urea breath test. Both methods rely on the direct detection of the bacterium or its activity, are expensive, and can cause discomfort to the patient. By contrast, a serological test with good sensitivity and specificity could be the first choice for diagnosis because of its lower cost and ease of use. The ADVIA Centaur® *H. pylori* IgG assay* is a chemiluminescent two-step magnetic microparticle-based immunoassay that utilizes an *H. pylori* bacterium lysate in the solid phase and an acridinium ester molecule in the lite reagent. The assay is designed for the qualitative detection of *H. pylori* IgG antibodies in human serum and plasma (EDTA and lithium heparin) to rapidly identify *H. pylori* infection in symptomatic populations. The objective of this preliminary study was to evaluate method comparison, precision, and onboard stability of a prototype automated *H. pylori* IgG assay tested on the ADVIA Centaur XP Immunoassay System.

Methods: Positive percent agreement (PPA%) and negative percent agreement (NPA%) for *H. pylori* IgG were assessed by testing 313 serum samples against the LIAISON *H. pylori* IgG (DiaSorin) or IMMULITE® 2000 *H. pylori* IgG assays (Siemens Healthineers.). The sample origin was as follows: 233 unselected samples from Banc de Sang i Teixits de Catalunya (Spain) and 80 intended-use samples from Cerba (France). Other relevant characteristics including a 5-day precision study (CLSI EP15-A3) and reagent onboard stability (CLSI 25-A) were also evaluated.

Results: Evaluation of the patient samples indicated that the observed PPA% ranged from 95.1 to 95.6% and from 95.9 to 97.1% when compared to the LIAISON *H. pylori* IgG and IMMULITE 2000 *H. pylori* IgG assays, respectively. The observed NPA% ranged from 95.4 to 96.2% (vs. LIAISON assay) and from 94.4 to 95.8% (vs. IMMULITE assay). The assay demonstrated good preliminary precision, with repeatability and within-laboratory %CVs between 2.0 to 5.0% and 4.0 to 5.9%, respectively. In addition, initial reagent onboard stability was verified up to 8 weeks.

Conclusions: The results of this study demonstrate good preliminary performance of the prototype ADVIA Centaur *H. pylori* IgG assay in terms of method comparison, precision, and onboard stability.

*Under development. The performance characteristics of this product have not been established. Not available for sale and future availability cannot be guaranteed.

B-077

Genotyping of Hepatitis C Virus by a Single-Tube, One-Step RT-PCR, Microarray Based Test

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Background:

Hepatitis C virus (HCV) is a significant medical problem worldwide, as acute infection is typically asymptomatic and 75-85% of patients develop chronic infection, which may lead to cirrhosis and hepatocellular carcinoma decades later if not treated. Hepatitis C virus (HCV) is divided into six major genotypes (1-6) with multiple subtypes. The identification of HCV genotype is an important predictor of the virologic response and is used to select the most appropriate treatment protocol. Commercial tests for HCV genotyping rely on sequencing, real-time PCR or line blot methodologies. The aim of this study is to use pre-characterized HCV specimens to evaluate a single-tube, one-step RT-PCR microarray based test for HCV genotyping.

Methods:

A total of 110 plasma specimens were pre-characterized using the Siemens Versant HCV Genotype 2.0 Assay. HCV RNA was extracted from the plasma samples using the MagnaPure 96 Instrument and sample results obtained with the microarray based test were compared. HCV RNA was amplified by a one-step RT-PCR method with thermal activated PCR primers. Fluorescent labelling of PCR products was achieved by primer extension prior to hybridization to a microarray chip.

Results:

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

Conclusions:

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

FOR RESEARCH USE ONLY

B-078

Exploring Potential Quasispecies Variants of Influenza H1N1pdm09 Virus

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

Methods and Results: We provided 10 Taiwanese H1N1pdm09 genomes isolated in the 2015-16 season by Sanger sequencing, including five severe (four patients had pneumonia, pleural effusion, and adult respiratory distress syndrome, and one had pneumonia and myocarditis), and five non-severe (four had upper respiratory infections, and one pneumonia case without other complications) cases. Influenza genome in approximately 13.5 Kb contained eight segments which included PB2, PB1, PA, HA,NP,NA, MP, and NS. In the 10 Taiwanese genomes, NA-74, -151, -314, and NS2-22 were detected, exhibiting more diversity than other positions. Only NA-151 showed residue "X" (as codon "RAC") and "D" ("GAC") in all 5 severe and 5 non-severe cases perfectly. This nucleotide ambiguity code "R" presented a dominant variant as "G" and a minor as "A". To estimate the prevalence of this minor variant, 7469 H1N1pdm09 genomes from 2009 to 2016 were downloaded from GISAID database. 94 translated nucleotide sequences exhibited non-D residues at NA-151, including 89"X", 4 "E", and 1 "N". Interestingly, increasing counts of X-residue in 2009, 2013, 2014, 2015, and 2016 were 1, 3, 4, 38, and 43, respectively. According to the prevalence of reported quasispecies signatures (HA-155, HA-222, and NA-275), we additionally identified 3 PB2, 1 PB1, 10 HA, 3 NA, 2 M2, 1 NS1, and 2 NS2 signatures in this study. **Conclusion:** This study began with focusing on exploring potential quasispecies variants in H1N1pdm09 genomes. For the 2015-16 outbreak season in Taiwan, mutation from Asp (D) to Asn (N) at NA-151 was detected in five severe cases, comparing to none in five non-severe cases. We also observed increasing prevalence of this variant from 2009 to 2016. This prevalence was under-estimated because the published sequences in database usually represent the dominant population of sequencing calls. This finding suggested that viral quasispecies at NA-151 was not a sporadic event and it might promote viral growth and further associate with disease severity. Subsequently, a total of 25 potential signatures were identified from 7469 genomes. In summary, these findings could help us to better understand the association of quasispecies variants and clinical outcome. More investigation is needed to demonstrate how those quasispecies signatures contributed to influenza outbreaks.

B-079

Analytical Validation of SAA Detection (O-SAA) by Particle-enhanced Immuno-turbidimetric Method

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Background:

Serum amyloid A (SAA) is an acute phase protein with a molecular weight of approximately 12kDa. In the acute phase reaction, SAA is synthesized in the liver and is regulated by interleukin-1 (IL-1), IL-6 and tumor necrosis factor (TNF). Similar to C-reactive protein (CRP), SAA is a sensitive indicator of inflammation and the concentration of SAA may rise up to 100-1000 times of its base value, which is helpful for inflammation diagnosis, and the initiation of the appropriate therapy. It has been shown that SAA levels increase significantly in viral infections, while the increase in CRP levels are less obvious. Thus, SAA is a sensitive and reliable indicator for evaluating viral infection. In order to demonstrate the usefulness of SAA in early infection, a point-of-care testing method for SAA in human serum, plasma and whole blood, using the O-SAA latex enhanced immunoturbidimetric kit on the Ottoman analyzer (Shanghai Upper Biotech Pharma Co.,Ltd, China) was studied.

Method:

Simultaneous samples of serum, plasma and whole blood were collected from 100 individuals. The serum samples were measured using a SIEMENS BN II specific protein analyzer with the corresponding N Latex SAA kit. The samples of serum, plasma and whole blood (in each case n=100) were analyzed using Upper's O-SAA regent kits on the Ottoman Specific Protein work-station.

Results:

A. Serum samples comparison

Serum samples (n=100) within the reportable range of O-SAA (5-288mg/l) were analyzed on both Ottoman and Siemens system. Linear regression analysis showed excellent correlation (R^2=0.9869) between the two systems with a slope of 1.0317 for SAA.

B. Serum and plasma samples comparison on the Ottoman system.

Linear regression analysis showed excellent correlation (R^2=0.9874) between the serum and plasma samples with a slope of 0.9891 for SAA.

C. Serum and whole blood samples comparison on the Ottoman system.

Linear regression analysis showed excellent correlation (R^2=0.9855) between the serum and whole blood samples with a slope of 1.0037 for SAA.

D. Analytical Concordance

Concordance analysis of SAA showed excellent analytical agreement between O-SAA and BNII with a sensitivity of 100%, specificity of 95.3%, and a concordance of 98% for SAA.

Conclusions:

SAA concentration detected by Upper's O-SAA regent kits correlated well with SIEMENS BN II specific protein analyzer and the corresponding N Latex SAA kit. The plasma and whole blood results obtained with the O-SAA kit compared excellently to the results for the corresponding serum samples. It is concluded that SAA values for serum, plasma and whole blood can be reliably determined using Upper's O-SAA regent kits on the Ottoman system. Whole blood can be used with the O-SAA kit, saving the centrifugation step and allowing faster access to patients' results. The O-SAA kit on Ottoman provides a reliable, rapid and quantitative test which takes less than 5 minutes and is especially useful in evaluating viral infection in emergency situations.

B-080

Peformance evaluation of LumipulseG HTLV-I/II

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

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

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

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

B-081

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

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

B-082

Performance Evaluation of the DxN Zika Virus Assay - EUA on the Beckman Coulter DxN VERIS Molecular Diagnostic System (EUA Version)*

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OBJECTIVE: Zika Virus became a global health crisis in 2016, leading to the FDA granting "Emergency Use Authorization" (EUA) regulatory status to certain Zika assays. In response, Beckman Coulter developed a qualitative molecular Zika assay on the DxN VERIS System. The objective of this study was to evaluate the performance of the assay.

METHODS: The DxN Zika Virus Assay - EUA is a fully automated Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay for the detection of Zika Virus RNA in human serum and EDTA plasma. The assay was evaluated for positive and negative percent agreement (PPA and NPA) via method comparison, sensitivity (Limit of Detection; LoD), sample matrix equivalency, cross-reactivity, and inclusivity. The PPA and NPA of the assay against the Roche LightMix® Zika rRT-PCR Test (EUA) were evaluated using a total of 215 clinical patient samples. Discordant samples were tested on the Hologic Aptima® Zika Virus Assay. To test LoD, a 5-replicate range finding study was first conducted to determine the tentative LoD, followed by a 20-replicate study near the tentative LoD. To test for sample matrix equivalency, 40 paired EDTA plasma/serum samples were evaluated at three target concentrations. Cross-reactivity was evaluated using *in silico* sequence alignments and by testing microorganisms, viruses or purified nucleic acid from the following: Dengue Types 1-4, West Nile Virus, St. Louis Encephalitis, Chikungunya, Mayaro Virus, Parvovirus, Plasmodium falciparum, and Yellow Fever Vaccine Strain. Inclusivity analysis of Zika virus strains was conducted by testing both contemporary and African-origin strains, and by performing a BLAST *in silico* analysis.

RESULTS: Evaluation of patient samples using the DxN Zika Virus Assay - EUA indicated a PPA of 96% (48/50) and the NPA ranging from 45% (25/55) to 100% (50/50 and 60/60) when compared to the Roche assay. The 30 discordant samples from the 45% NPA set were evaluated on the Hologic assay; 24/30 of these samples were positive in disagreement with the Roche assay. The LoD was determined to be 126 copies/mL. This compares well to the reported LoDs of the Roche assay (181 copies/mL) and the Hologic assay (5.9 copies/mL). LoD testing was also performed using FDA Zika reference material and was determined to be 200 RNA NAAT Detectable units/mL for Sample S1 and 500 RNA NAAT Detectable units/mL for Sample S2. Serum and EDTA plasma matrix equivalence was demonstrated across all sample concentrations. No cross-reactivity to other microorganisms was detected in the assay. The assay was determined to be specific to contemporary strains of Zika since African-origin Zika isolates tested were not detected at 1.5xLoD. *In silico* inclusivity analysis showed the contemporary strains had ≥95% homology with the assay primers and probe, while the African-origin strains showed lower (82% to 96%) homology.

CONCLUSIONS: Beckman Coulter's DxN Zika Virus Assay - EUA demonstrates good performance with respect to positive and negative agreement, sensitivity, sample matrix equivalency, cross-reactivity, and inclusivity.

*DxN Zika Virus Assay - EUA and DxN VERIS Molecular Diagnostics System (EUA version) have been submitted to the U.S. FDA for Emergency Use Authorization. Not available for distribution in the U.S.

B-083

Clinical Performance of Elecsys® HBsAg II in Subjects with Increased Risk of Hepatitis

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

Abbott ARCHITECT HBsAg and HBsAg confirmatory reference testing was performed at two US sites. Final HBsAg interpretation was based on the confirmatory testing of repeatedly reactive samples.

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

Cohort	Positive		Negative	
	n	Percent Agreement, CL	n	Percent Agreement, CL
Adult	39	100.00% 90.97-100.00%	2020	99.75% 99.42-99.92%
Supplemental[1]	391	99.23% 99.77 -99.84%	6	83.33% 35.88-99.58%
Pediatric	0	NA NA	128	100.00% 95.61-99.68%
Pregnant	13	100.00% 75.29-100.00%	205	100.00% 98.22-100.00%
[1]Population to enhance prevalence of acute and chronic hepatitis B subjects				

Two hundred and sixty-nine specificity specimens representing 21 diseases demonstrated agreement of 99.33% to 100.00%. Additionally samples spiked with bacterial and viral extracts demonstrated concordance with un-spiked control.

Seroconversion sensitivities in test and reference assays were equivalent in thirteen of fourteen commercial panels tested; Elecsys HBsAg II converted one draw later in one panel. Imprecision (CLSI EP5-A3) was evaluated using three reagent lots in three US sites. Three replicates of imprecision pools were tested in two runs per day for five days. Repeatability %CV values for pools (mean COI ≥0.70) ranged from 2.3 to 4.6%. %CVs for Reproducibility ranged from 3.4 to 6.0%. The C5–C95 interval around assay cut-off (CLSI EP12-A2) ranged from 0.93 to 1.06 COI.

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

B-084

Update on "Strategies for Improving Rapid Influenza Testing", the New Spanish Version, and "Influenza Preparedness and Response" Courses for Clinicians

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Objective: To enhance the knowledge of clinicians about rapid influenza testing (RIDT) and pandemic preparedness by offering free online courses, "Strategies for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS)" and "Influenza Preparedness and Response in Ambulatory Settings" with continuing education credits. Further objectives were to gather feedback from participants about the courses and other educational needs. Based on that feedback, and the need to expand outreach to participants whose primary language is Spanish, a new Spanish version of SIRAS was developed. **Relevance:** RIDTs are the most widely used screening tests for influenza and are often used for making treatment decisions, despite concerns about their variable predictive value. A survey conducted by the CDC and TJC in 2012 identified the need for education on the use of RIDT, interpretation of results, and influenza pandemic preparedness. Feedback from these courses, identified the additional need for a Spanish version of SIRAS. **Methods**: The SIRAS course was developed and launched in Oct. 2012 (http://www.jointcommission.org/siras.aspx). The pandemic preparedness course (http://www.jointcommission.org/topics/influenza_pandemic_preparedness.aspx) was developed and launched in Apr. 2015. 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 are offered with both courses. Courses are updated annually and relaunched in Oct. of the respective year. In 2016 the SIRAS course was translated into Spanish. TJC uses multiple media channels to market these courses. Course utilization data are compiled annually; e-resource utilization is tracked cumulatively. **Validation**: Since 2012, there were 10,785 unique visitors to the SIRAS webpage, 4,015 unique visitors to the Pandemic Preparedness webpage, and 186,795 views of specimen collection videos. In 2016, 80% (535/668) of participants completed a voluntary SIRAS course evaluation; 33% said the course validated current practices and 29% planned to change practices based upon course content. Of those planning to change practices and describing which practices (119/153), 18% planned to change influenza surveillance practices, 13% infection control practices, 11% influenza diagnostic testing practices, and 18% planned to take the influenza preparedness and response course, offered by CDC and TJC, to improve pandemic planning. Of the participants who evaluated SIRAS in 2016 (n=535), 99% found the course useful, 94% had a better understanding of RIDT, and 89% would recommend the course to others. The Spanish version of SIRAS was launched in Oct. 2016. In 2016, 25% (265/1072) of participants completed a voluntary Pandemic Preparedness course evaluation. Of participants who planned to change practices and described the changes (188/263), 25% planned to develop or change preparedness response plans, 37% to improve communication between their practices and local/state health departments, and 34% to improve specimen collection procedures. Of those completing the course evaluation in 2016 (n=265), 86% were satisfied overall and 82% were likely to recommend the course to others. **Conclusions**: The ongoing opportunity for continued education in influenza testing and preparedness was welcomed by participants. Annual updating of courses before the onset of influenza season attracts increased usage of e-resources and enrollments to the course. Specimen collection videos, and other e-tools continue to be popular.

B-085

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

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

Methods: We compared CMS Medicare outpatient claims (OTP) data with positive test volumes using rapid flu tests from the CDC National Respiratory and Enteric Virus Surveillance System (NREVSS) as the "gold standard" for flu activity. We compared both V_dx and V_test to the gold standard for each of 10 selected states (one per HHS region). Spline models were developed and applied to fit the time series of weekly volumes of V_dx, V_test, and NREVSS test positives (dependent variables) for 5 influenza seasons (2007 to 2012), with the week number as the independent

variable. Using the fitted models, we calculated predicted weekly volumes and standard deviations (SD) for V_dx and V_test. Criteria for an initial alert were defined as: 1. an increase of the predicted volume from the previous week by two SD (2 SD criterion), or 2. an increase of the predicted volume from previous week by 0.45 SD for two consecutive weeks (0.45 SD criterion). A flu "episode" was defined as the interval between an initial alert and a drop in volume meeting the same criterion.

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

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

Conclusions: This study demonstrated the potential use of CMS claims data for early detection of increasing influenza activity in specific regions of the U.S. In this retrospective analysis, spline models performed well for early detection of an increase in flu activity. The 0.45 SD criterion demonstrated greater sensitivity over the 2 SD criterion yielding earlier detection by an average of over three weeks than predictions using the gold standard data. The consecutive week exceedance requirement using 0.45 SD, which required two consecutive changes in the same direction to identify a change in activity, reduced the occurrence of "false peaks" and resulted in reasonably manageable positive predictive values. In our study, V_test was equally effective to V_dx for identifying influenza activity in this convenience sample. In this setting, neither test results nor diagnosis were absolutely necessary for surveillance using CMS data.

B-086

Performance Evaluation of the ROCHE E 170 for the Determination of Procalcitonin in blood.

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

Methodology: Roche Elecsys BRAHMS procalcitonin assay is a sandwich assay with a total incubation of 18 minutes. The assay is **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" with 2 incubations; results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

We evaluated: the sensitivity, linearity, within assay coefficient variation for replicates from synthetic materials and from patient sample, between assay coefficient of variation, reportable range, and correlation was done using reference laboratory. Statistical analyses were done using Analyse-it.

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

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

B-087

New Molecular Diagnostic Assays for the Detection of *Chlamydia trachomatis*/*Nisseria gonorrhoeae and Trichomonas vaginalis* on the DxN VERIS Molecular Diagnostics System

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OBJECTIVE: Beckman Coulter is currently developing assays for the detection of sexually transmitted diseases on its DxN VERIS System including *Trichomonas vaginalis* (TV) and a multiplex assay for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). CT and NG may produce asymptomatic infections, especially in women, and if left untreated, can lead to serious reproductive and other health problems with both short-term and long-term consequences. TV, which colonizes in both men and women, is a protozoan parasite responsible for causing trichomoniasis. The DxN CT/NG and DxN TV Assays are qualitative *in vitro* diagnostic assays for the detection of DNA from theseorganisms in male/female urine, vaginal (patient and clinician collected) and endocervical swabs, and PreservCyt® specimens from symptomatic and asymptomatic individuals. The two assays use magnetic particle extraction and TaqMan® PCR technology on the DxN VERIS platform to detect specific gene sequences of CT/NG and TV. **METHODS:** The DxN CT/NG and DxN TV Assays are designed to detect DNA in a sample collection tube with a pierceable cap. Performance of the two assays was evaluated for analytical sensitivity and specificity. These evaluations included limit of detection, precision and reproducibility, potential interfering substances, and cross reactivity. Study methods were based on Clinical and Laboratory Standards Institute (CLSI) Guidelines. Additionally, the DxN CT/NG and DxN TV Assays were evaluated against two comparator assays with clinical samples. Sensitivity and specificity to the patient infected status (PIS) was calculated. PIS was determined to be positive if two comparator molecular assays produced positive results. PIS was determined to be negative if the two comparator assay produced negative results. If the two comparator assays produced discrepant results, a tie-breaker comparator assay was run to determine the PIS. **RESULTS AND CONCLUSIONS:** Both DxN TV and the DxN CT/NG assays have a turn-around time from sample to result of ≤ 75 minutes. Evaluation of clinical samples across specimen types demonstrated that the DxN CT/NG assay was 96 to 100% sensitivity and 97 to 100% specificity when compared to the PIS for the CT analyte. DxN CT/NG achieved a LoD of 10 to 25 EB/mL for CT Serovars D and H and 5 CFU/mL for NG strains 19424 and 49226. The assay produces reproducible results and demonstrated good precision with a CV of 1-2 % at 3XLoD levels for both organisms. The DxN TV Assay demonstrated a LoD of 1.5 to 4.5 TV/mL with the Metronidazole-susceptible (30001), and Metronidazole-resistant (50143) strains of TV depending on the sample type. Evaluation of clinical samples on the DxN TV assay achieved 97% to 100% sensitivity and 95% to 98% specificity when compared to the PIS. The assay produces reproducible results and demonstrates good precision with a CV of 1.2 to 1.7% at 1X and 3X LoD. *DxN CT/NG and DxN TV Assays are in development. DxN Veris product line has not been submitted to U.S. FDA and is not available in the U.S. market. DxN VERIS Molecular Diagnostics System is also known as VERIS MDx Molecular Diagnostics System and VERIS MDx System.

B-088

Burkholderia Cepacia Outbreak in Long-Term Care Facilities.

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Background: *Burkholderia Cepacia, a gram negative bacilli,* is a group or complex of bacteria that can be found in soil and water. It is more colonizing bacteria than infecting bacteria; however it may cause problem if found in people with weak immune system, cystic fibrosis, or if found in sterile body fluid. A recent multistate outbreak of *Burkholderia Cepacia* in blood was reported due to contaminated prefilled saline flush was reported. A majority of the cases are reported in long-term care facilities or rehabilitation facilities.

Methodology: During the months of 2016 in which the contaminated saline was used in Long-Term Care Facilities, 1276 sets of blood cultures collected from the residents. Every set included two vials (aerobic and anaerobic) which were incubated in a Bactec instrument. Positive cultures were subcultured and then identified using Microscan96 Walkaway conventional panels. Data analyses were done for all the facilities and then isolating the facilities with positive isolate for *Burkholderia Cepacia*. Statistical analyses were done using Analyse-it.

Results: 15.4% of the total blood cultures were positive. *Burkholderia Cepacia* accounted for 12.8% of these positive cultures, and was found only in 7 facilities, accounting for127 of the 1276 cultures tested. Of these 127 patients, 28% of them had a positive blood culture. Of those positive cultures 71.4% of the bloodstream infections were due to *Burkholderia Cepacia*. No deaths to our knowledge were reported due to *Burkholderia Cepacia* in the identified patients.

	All Facilities	Affected facilities
% positive blood culture	**15.4%**	**28.0%**
% B. Cepacia/all blood culture	**2.0%**	**19.7%**
% B. Cepacia/total positive blood culture	**12.8%**	**71.4%**

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

B-089

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

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

Methods: The VERSANT® HCV Genotype 2.0 assay (LiPA) is a line probe assay that identifies HCV genotypes 1-6 and subtypes a and b of genotype 1 in human serum or plasma specimens. Using reverse hybridization technology, biotinylated DNA PCR product generated by RT-PCR amplification of the 5' UTR and core regions of HCV, RNA is hybridized to immobilized oligonucleotide probes.

Analytical validity and clinical validity studies were performed at three clinical trial sites to validate the performance of the VERSANT HCV Genotype 2.0 assay (LiPA). The genotyping rate (GR) and genotyping accuracy (GA) were evaluated for each HCV genotype/subtype in order to assess the integrity of the assay results compared to the NS5b reference method. Results were interpreted manually and using the semi-automated method.

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

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

In the clinical validity study, the SVR_{12} rate for all patients tested was 88.5% (192/217) for both methods. For individual genotypes/subtypes, the observed SVR_{12} rates ranged from 72.5 to 100%. Results indicate patients diagnosed with genotype 1 had significantly lower SVR_{12} rate compared to pooled non-1 genotype patients. The relationship between the HCV genotype/subtype and the SVR_{12} rate is essential for achieving successful clinical outcomes in chronically infected HCV patients treated with direct-acting antiviral (DAA) regimens.

Conclusions: The VERSANT HCV Genotype 2.0 assay (LiPA) provides interpretable and accurate diagnosis of HCV genotypes 2, 3, 4, 5, and 6 and subtypes 1a and 1b as shown by an overall GR and GA rate of ≥88% and ≥99%, respectively, in both studies. SVR_{12} rate by genotype/subtype is consistent with published results using similar treatments with DAAs.

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

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

B-090

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

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Introduction:

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

Objective:

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

Results:

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

Conclusions:

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

B-091

Panel of Zika virus infection diagnosis.

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

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

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

Results Majority of patients were women (60; 78%), average age was 35.8. Seventy-three patients were non-reactive by PCR from blood (table 1).

Only one sample presented positive PCR on urine. At the time of panel sample collection, 18 samples (23%) were IgG positive.

Table 1. PCR and ELISA serology results among 78 patients, Brazil, 2016.

		PCR urine		ELISA_IgM		Elisa_IgG	
		Positive	Negative	Positive	Indet/ Negative	Positive	Indet/ Negative
PCR blood	**Negative (N=73)**	1	72	6	67	18	55
	Not done (N=5)	0	5	0	5	0	5
TOTAL (%)		1 (1.3%)	77 (98.7%)	6 (7.7%)	72 (92.3%)	18 (23.1%)	60 (76.9%)

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

B-092

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

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

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

B-093

Zika virus RT-PCR performed in urine and plasma.

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Background. Zika virus (ZIKV) infection causes a transient viremia and the diagnosis by RT-PCR has been most successful within one week after the onset of clinical illness. The practical guidelines have recommended search for ZIKV-nucleic acid (RNA) on blood and urine. ZIKV RNA can be detected longer in urine than in serum, extending the period that a definitive diagnosis of ZIKV infection can be established by RT-PCR. The objective is analysis pairs of RT-PCR on urine and blood to assess the positivity of the test on these two different clinical specimens.

Methods. During 2016 we evaluated 257 samples of urine and plasma underwent to PCR ZIKA virus (ZIKV) determination on the same day.

Results. A total of 12 samples of blood (4.7%) and 24 samples of urine (9.3%) were positive for ZIKV RT-PCR. The majority of samples (84.8%) were negative for ZIKV RT-PCR on both blood and urine. Seven samples were negative RT-PCR on urine but positive RT-PCR on blood; and 19 samples were negative RT-PCR on blood but positive on urine.

		Urine		
		Positive (N=24)	Negative (N=226)	Inconclusive (N=7)
Blood	Positive (N = 12)	4	7	1
	Negative (N= 243)	19	218	6
	Inconclusive (N=2)	1	1	0

Conclusions. Although the ZIKV-PCR is detectable on the blood within the first week of clinical illness and is detectable on the urine up to 21 days after onset of symptoms, we failed to demonstrate a clear superiority of RT-PCR on urine compared to the blood. However, the RT-PCR on blood allowed the diagnosis of 3% of the patients that the ZIKV infection would not be recognized if PCR was performed only in urine, and the RT-PCR on urine allowed the diagnosis of 7% of the patients that ZIKV infection would not be recognized if PCR was performed only in the blood.

B-094

Identification of *Non-Tuberculosis Mycobacterial* species by DNA sequencing: an experience from an endemic tuberculosis country.

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Background. *M. tuberculosis* disease remains a major international health threat causing million of cases and deaths yearly in the world. Reported incidence of nontuberculosis mycobacterial (MNT) disease, however, appears to be increasing worldwide, particularly among higher risk group, such as immunocompromised hosts. Rapid and accurate differentiation of mycobacterial species is important to guide clinical management and appropriate therapy. The accurate identification of NTM, however, is becoming more problematic, mainly because the increased number of species, and their fastidious and low growth rate. In this context, molecular diagnosis and typing is a valuable tool and commercial platforms for that are available. We reported here the distribution of Mycobacteria species isolated by culture and submitted to genotyping from Jan to Dec 2016.

Methods. Positive mycobacteria cultures were boiled at 100oC for 30 minutes to obtain DNA which was submitted to PCR amplification and amplicon sequencing using two targets: hsp65 (450 bp) and RPOb (764pb).

Species assignment was performed by sequence alignment and comparison employing BLAST tool (blast.ncbi.nlm.nih.gov).

Results. In 2016 a total of 56 positive mycobacterial cultures from 55 patients were sequenced and the results are summarized in tables 1 and 2. One patient had two different Mycobacteria species isolated from different clinical species (*M. mageritense* from breast secretion and *M. fortiutum* from respiratory sample) isolated in samples collected at one-month interval.

M. tuberculosis was the most prevalent species identified (23%), followed by *M. kansasii* and *M. abscessus* (16% each). *M. tuberculosis* and *M. kansasii* were the most prevalent species, with 6 cases each. Among 25 respiratory samples, MNT was identified in 76% of the cases.

Table 1. Mycobacteria species identified among 56 positive samples.

Mycobacterium species	< 18 y	> 60 y	18 - 60 y	Total
Mycobacterium abscessus		4	5	9
Mycobacterium avium		3	1	4
Mycobacterium Kansasii		3	6	9
Mycobacterium tuberculosis	1	5	7	13

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

B-095

Biological Stability Study on the 1st Korea National Standard for anti-Human Immunodeficiency Virus Type 2

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Background: Infection with HIV-2 was mainly confined to western Africa, but the number of people infected with the HIV-2 has recently increased in Europe, India, and the United States. According to the guidelines for HIV testing revised by the Centers for Disease Control (CDC) in the US in 2014, patients who are positive in HIV screening tests should take a further test that differentiates HIV-1 from HIV-2. Thus, the development of products for differentiations of HIV-1/2 is expected to increase in the future. At the same time, it is expected to increase demands for HIV-2 standard material required to develop and approve new products and to carry out quality control.

Methods: The 1st Korea National Standard for anti-HIV-2 which is composed of 2 kinds of mixed titer panels consisting of one negative and five positive samples each, has been established in 2015 using plasma source collected and transferred from Togo in Africa. Mixed titer panels for anti-HIV-2 have been characterized with various assays differentiating variable degree of cross-reactivity between HIV-1 and HIV-2. Biological stability has been evaluated with real-time stability assessments and accelerated degradation test (ADT) at various temperature conditions such as 4°C, 20°C, 37°C, and 45°C for 6 months by analyzing at certain intervals. The results were statistically compared with initial values. *Results:* HIV-2 positivity is confirmed by Geenius HIV 1.2 Confirmatory Assay (Bio-Rad Laboratories, Hercules, CA), New LAV BLOT HIV BLOT II (Bio-Rad Laboratories, Hercules, CA), INNO-LIA HIV I/II SCORE (Fujirebio Europe, Gent, Belgium), MP diagnostics HIV BLOT 2.2 (MP Biomedicals, Solon, OH, USA), and SD BIOLINE HIV 1/2 3.0 and cross-reactivity with HIV-1 is evaluated with New LAV BLOT HIV BLOT I (Bio-Rad Laboratories, Hercules, CA), Cambridge Biotech HIV-1 and Serodia HIV-1 PA (Fujirebio Diagnostics, Inc., Japan). HIV-2 RNA is also identified using in-house real-time PCR. As a result of real-time stability, it has confirmed that the antibody titers of the 1st Korea National Standard Panels for anti-HIV-2 established in 2015 are still valid and maintained well. According to the ADT results, the stability was maintained at 4°C for 6 months, slightly decreased but still maintained at 20°C for 6 months, and maintained at 37°C and 45°C for 1 month, when comparing permanently maintained at -80°C. Ten consecutive freeze-thaw cycles and storage of specimens at -20°C and 4°C for 30 days resulted in no loss of anti-HIV-2 reactivity nor false positive samples. *Conclusions:* The 1st Korea National Standard for anti-HIV-2 is an exclusive reference material and will be very useful to resolve cross-reactivity with HIV-1 and to rule out the possibility of co-infection, especially for the accurate profiling of the antibodies. Acknowledgement:This research was supported by a grant (17172MFDS339) from Ministry of Food and Drug Safety and Human Serum Bank (NRF-2015M3A9B8030780) through of the MSIP, Korea

B-096

Does Syphilis Chemiluminescent Microparticle Immunoassay(CMIA) correlate with Treponemal Pallidum Agglutination Assay(TPPA)

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Background:

Syphilis testing is complicated and requires special algorithms in testing. We reviewed syphilis screening using chemiluminescent microparticle immunoassay (CMIA) against confirmatory treponemal pallidum passive particle agglutination assay (TPPA) and analysed discordant results.

Methods:

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

Results:

470 (2.49%) results were>1.0. The median age for CMIA signals>1.0 and <1.0 were 44 and 35 years old, while their female:male ratio were 1:3 and 3:1 respectively.

Out of 470 results with CMIA signal >1.0, 74 were non-reactive, 10 were indeterminate and 386 were reactive on TPPA, with 84% sensitivity. Their median CMIA was 1.62, 1.62 and 17.88, while their median age were 36, 64 and 47 years old respectively. Of 386 TPPA reactive results, 167 were RPR negative, and median RPR titre was 4 for RPR positive results.

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

Conclusions:

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

B-097

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

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

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

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

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

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

	Case 1	Case 2	Case 3	Case 4
Age	46	41	63	54
Sex	Female	Male	Male	Female
Time from KT (years)	1.7	6.6	4.4	3.0
DEN diagnosis based on	IgM +	IgM +	IgM +	IgM +
Checked IgM-DEN serology	1.0	0.7	**2.2**	0.8
Checked IgG-DEN serology	**5.4**	**5.2**	**6.8**	**4.2**
Checked IgG-ZIK serology	0.5	0.51	**2.7**	0.1
Clinical picture	Asthenia + myalgia	Fever + headache + myalgia + retroocular pain	Fever + myalgia + chills	Fever + asthenia + vomiting + diarrhea
Outcome	Recovery	Recovery	Recovery	Recovery
KT, kidney transplant.				

Conclusions.

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

B-098

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

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

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

Results. Throughout 2016 a total of 1,544 of CD toxin test were performed from 1,255 patients; 210 patients performed more than one test (average 1.2, range 2 - 6). The general positivity rate was 16% (253/1542) and 2% of samples had indeterminate results. A total of 10 patients presented B1/NAP1/027 positive strain.

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

B-099

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

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

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

Results: The sensitivity and specificity of AFIAS anti-HCV assay were 99.8% (95% CI: 97.1-99.6%) and 99.1% (95% CI: 98.3-99.6%), respectively. The kappa value for the agreement between two assays' results was 99.0% (k=0.975, 95% CI: 0.962-0.987). AFIAS anti-HCV assay detected all samples with genotype 1, 1a, 1b, 2a, 2b, 4 and 6. Evaluation with 10 seroconversion panels demonstrated the adequate sensitivity. There was no interference or cross-reactivity with other infection, anticoagulant, pregnant-associated, icteric and hemolysis samples. Inter-assay CVs ranged from 4.4 to 5.4% and lot-to lot variation ranged from 3.5 to 6.6%.

Conclusion: The AFIAS anti-HCV assay demonstrated acceptable analytical performance and diagnostic accuracy that was equivalent to current automated chemiluminescent immunoassay. It can be very useful in emergency or small clinical laboratory for the detection of anti-HCV due to its simplicity and flexibility.

B-100

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

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

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

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

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

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

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

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

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

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

Conclusions: Multiplex assay allows the detection of the three enteropathogens in only one reaction, which reduces the cost and is less consuming .For *Salmonella, Shigella/EIEC* and *Yersinia, "*VIASURE Multiplex Real Time PCR Detection Kits" are highly sensitive and specific, being comparable in sensitivity and specificity to culture method and RIDA®GENE Kits.

In the diagnosis of *Campylobacter*, "VIASURE Multiplex Real Time PCR Detection Kits" are the most sensitive one.

Molecular techniques have the additional advantage that detects unusual varieties of *Campylobacter* species in comparison to culture method.

B-101

Molecular diagnosis by Real-Time PCR to detect emerging viruses Dengue, Zika, Chikungunya and West Nile Virus

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Background: The rapidly expansion across the globe of the more frequent illnesses caused by arboviruses such as Dengue, Zika, Chikungunya and West Nile Virus and their similar clinical presentations made necessary a proper diagnostic. Dengue virus (DENV) is the most common tropical virus infection and is caused by four distinct serotypes of the Dengue virus (DEN-1, DEN-2, DEN-3 and DEN-4). Dengue serotyped is required to the treatment of patients, control of DENV outbreaks and transmission-blocking strategies targeting the vector, as well as for the development of vaccines and antivirals. The aim of this study is to compare Real-Time PCR assays with the routine diagnosis technique as well as to serotype Dengue positive samples.

Material/methods: We performed a retrospective study where we tested 30 samples (28 serum and 2 urine samples), from August 2013 to August 2016. The patients presented typical symptoms for previous named arboviruses. Total genomic RNA was isolated from serum and urine samples with the "QIAamp Viral RNA Mini Kit"(Qiagen). Nucleic acids were amplified on thermocycler AriaMx (Agilent Technologies) using the monoplex assay "VIASURE *West Nile Virus* Real Time PCR Detection Kit" and the multiplex assay "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit"(Certest Biotec S.L) in comparison to Real-Time PCR assay "RealStar® Dengue RT-PCR kit 2.0" (Altona). Dengue serotyping was performed using the multiplex assay "VIASURE *Dengue Serotyping* Real Time PCR Detection Kit" (Certest Biotec S.L).

Relative to the routine diagnosis method, all samples were tested with inmunochromatography by "SD BIOLINE Dengue IgG/IgM (Standard Diagnostics)" and also with enzyme- inmuno assays using "Dengue ELISA IgM CAPTURE (Vircell)".

Results: 8/29 samples were positive for Dengue by multiplex Real-Time PCR assays, these results were confirmed by Altona. 1/29 was positive for Zika by VIASURE multiplex assay, being supported by serology and clinical diagnosis. No positive samples for Chikungunya or West Nile Virus were found by VIASURE Real-Time PCR assays.

Considering the phase of the disease, there is a total concordance between serological and Real-Time PCR results.

The positive samples for Dengue were serotyped. 3/8 belong to serotype Dengue 3, 3/8 samples were Dengue 2, 1/8 samples was Dengue 1 and 1/8 was not positive for DEN-1 to DEN-4.

Conclusions: A total concordance between serological results and Real-Time PCR has been found in this study. Molecular diagnosis is more accurate during first infection phase, being serological diagnosis proper during convalescence phase.

The multiplex assay "VIASURE *Dengue Serotyping* Real Time PCR Detection Kit" allows the Dengue serotyping, which, in combination with clinical and epidemiological risk factors provides an aid in the patient management.

B-102

Evaluation of the Diagnostic Value of Volume, Conductivity and Scatter Parameters Determined by Unicel DxH800 Coulter Cellular Analyzer in Sepsis in Comparison to Chronic Inflammatory and Nonsystemic Infection Cases

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Background: Sepsis is a systemic inflammatory response and clinical criteria for organ dysfunction are defined as an increase of 2 points or more in the sequential organ failure assessment (SOFA) score. Laboratory parameters can provide valuable information for the diagnosis and some investigations showed that volume-scatter-conductivity (VCS) parameters of cell counters might be beneficial. However, these parameters are reflections inflammatory status and chronic inflammatory states may cause interferences. Our aim was to evaluate diagnostic significance of VCS parameters in sepsis in comparison to rheumatoid arthritis (RA) and nonsystemic infection cases (NSI).

Methods: The study was conducted in Marmara University Pendik E&R Hospital Biochemistry Laboratory. Sepsis (n=22) (diagnosed according to the 2016 Sepsis Consensus Report), RA (n=68), and sputum culture positive respiratory tract infection patients (n=21) were enrolled. Blood samples for cell count and serum markers were collected on the same day of culture collection. VCS parameters were measured by Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA). Hs-CRP levels were determined nepholometrically and procalcitonin concentrations measured by electrochemiluminescence immunoassay. For Bacterial diagnosis; sputum samples were stained by Gram stain and examined immediately to analyze the quality of the samples. Qualified samples were inoculated on 5% sheep blood agar, Chocolate Agar, Haemophilus Agar, and MacConkey Agar (BioMerieux, France) by streaking method. After overnight incubation, the samples were evaluated.**Results:** The mean age was 64±15.5 years in sepsis patients, which was not significantly different than other groups. WBC counts were not significantly different. Procalcitonin and hs-CRP levels were significantly high in sepsis group in comparison to RA and NSI. Mean neutrophil volume (MN-V-NE) was the highest in the sepsis group (165±22.3) compared to that in the NSI (144±13.5) and RA (144±10) groups (P=0.002 and P=0.001, respectively). Mean neutrophil SD (SD-V-NE) was higher in the sepsis group (22.2±4.56), which was significantly different than RA group (17.5±3.6, P=0.001). For neutrophils, mean values for median-angle light scatter (MN-MALS-NE), lower-median-angle light scatter (MN-LMALS-NE) and low-angle light scatter (MN-LALS-NE) were significantly lower in the sepsis group. Mean monocyte volume was the highest in the sepsis group (190±15.4) compared to that in the RA (166±8.4) and NSI (169±8.74) groups (P<0.001 and P<0.001, respectively). Mean monocyte volume SD, mean axial light loss (M-AL2) and SD-axial light loss (SD-AL-2) significantly differentiates sepsis from other groups. ROC curves evidenced excellent sensitivity especially in the neutrophil parameters. MN-V-NE at 151, the sensitivity 81.8% and specificity 84.1 %; SD-V-NE at 18.3, the sensitivity 86.4% and specificity 81.8%; MN-MALS-NE at 129, the sensitivity 87.5% and specificity 72.7%; MN-LMALS-NE at 127, the sensitivity 87.5% and specificity 86.4%; SD-V LE at 72.5, the sensitivity 91% and specificity 81.1 %; MN-V LE at 175.5, the sensitivity 81.8 % and specifity 85.2; SD V MO at 21.7, the sensitivity 72.7% and specificity 81.6 %; MN-C MO at 127.5, the sensitivity 72.7% and specificity 81.5 % are achieved in our study.

Conclusion: We concluded that VCS parameters might be promising for differentiating between sepsis and non-sepsis cases. Additionally, they are obtained routinely, without any additional cost and time requirement thus making their prospects very promising.

B-103

Performance Evaluation of the ADVIA Centaur anti-HCV Assay* in the Pediatric Population

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Background: The HCV antibody immunoassay is clinically useful for managing cases of viral hepatitis infection in the pediatric population. This study assessed the performance of the ADVIA Centaur® anti-HCV assay (Siemens Healthcare Diagnostics Inc.) in a pediatric subpopulation consisting of children and adolescents presenting with signs and symptoms of hepatitis or at risk for hepatitis C virus (HCV) infection.

Methods: Concordance of the ADVIA Centaur anti-HCV assay* was assessed to a comparator assay commercially available in the U.S. and approved for use in children aged ≥10 years old. Serum samples from suspected or high-risk pediatric populations were tested in singleton on both assays. Samples with initial equivocal test results were retested in duplicate, and the results were interpreted as recommended by the assay manufacturers.

Results: A total of 55 subjects were included, aged 2 to 20 years old with a male/female ratio of 57.4/42.6%, respectively. The subjects' specimens yielded the following final results on the comparator assay: 1 equivocal, 6 reactive, and 48 nonreactive results. When excluding the equivocal specimen from the concordance analysis, the percent positive and negative agreements were 100.00% (95% CI 54.07 to 100.00) and 100.00% (95% CI 92.60 to 100.00), respectively. When including the equivocal specimen in the concordance analysis and counting this specimen as a discordant result for the positive agreement, the percent positive and negative agreements were 85.71% (95% CI 42.13 to 99.64) and 100.00% (95% CI 92.60 to 100.00), respectively. All positive anti-HCV results were confirmed to also be positive under RIBA testing.

The specimen which tested equivocal on the comparator assay tested negative on both the RIBA test and the ADVIA Centaur anti-HCV assay.

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

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

B-104

Performance Evaluation of the ADVIA Centaur HBc Total* and HBc IgM* Assays in the Pediatric Population

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

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

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

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

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

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

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

B-105

Comparison of two algorithms for the diagnosis of syphilis

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

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

Results: : In traditional syphilis screening algorithm, thirty eight patient samples were positive in RPR test, among which 32(84.2%) were positive in TPAb and 1 of 32(3.1%) was negative in ADVIA Centaur Syphilis. The rest six (15.8%) were TPAb negative. Eleven RPR(-) samples (6.7%) were positive in TPAb and 5 among them were positive in ADVIA Centaur Syphilis as well. Thirty six patient samples were ADVIA Centaur Syphilis (+) in reverse syphilis screening algorithm. Thirty one of them were RPR(+)/TPAb(+). The rest 5(13.9%) were RPR(-)/TPAb(+). Only one of 7 ADVIA Centaur Syphilis(-)/RPR(+)(14.3%) was TPAb(+). Analytical sensitivity and specificity of the traditional syphilis screening algorithm were 81.6% and 96.9%, respectively and those of the reverse syphilis screening algorithm were both 100%. **Conclusion:** The diagnostic performance of reverse syphilis screening algorithm was superior to the traditional syphilis screening algorithm in this study.

B-106

Performance of the VITROS® Prototype HTLV I/II Assay*

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Background / Objective:

HTLV-I and HTLV-II are closely related type C retroviruses. HTLV-I is etiologically associated with adult T-Cell leukemia, demyelinating disease, and degenerative retinal disease. HTLV-II has not yet been conclusively established as an etiologic agent for any specific disease. HTLV-I is endemic in some Caribbean countries, Southern Japan, and some areas of Africa and South America. HTLV II is endemic in several American Indian tribes. Transmission of HTLV I/II occurs via sexual contact, transfusion exposure to infected cellular blood components, intravenous drug abuse, or through breast milk. Routine screening of anti-HTLV-I/II is mandatory in many countries. We have assessed the performance of a prototype HTLV I/II Assay* on VITROS Immunodiagnostic Systems. This assay qualitatively detects antibodies to HTLV I/II in human plasma and serum and is suitable for donor screening or as an aid in the diagnosis of HTLV infection.

Method:

Antibody detection in the VITROS HTLV I/II Assay* is achieved using recombinant p21 and p24 HTLV I/II antigens coated on microwells which capture HTLV antibodies present in the sample. After addition of the HRP-labeled p21 and p24 HTLV I/II antigens 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 reagents on a VITROS 3600 Immunodiagnostic System with the exception of the dilutional 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 1668 fresh serum and plasma blood donor samples. Assay sensitivity was evaluated by testing 151 HTLV-I and 69 HTLV-II serological presumed positive patient samples and 1 commercial performance panel. Results from 4 serially diluted positive patient samples (2 HTLV-I and 2 HTLV-II) were compared to results from the AVIOQ HTLV-I/II Microelisa System run on the ORTHO VERSEIA Integrated Processor. Total within lab precision was evaluated over 15 days in accordance with CLSI EP05-A3 using 1 VITROS 3600 and 1 VITROS ECiQ Immunodiagnostic System.

Results:

The assay specificity was 100 % (1668/1668; 95%CI: 99.78 - 100 %) for donor patient samples. The mean S/C for these samples was 0.055 with 1/1668 samples > 0.4 S/C. The assay sensitivity was 100% (212/212; 95% CI: 99.28 - 100.0%) with the presumed positive patient panel. The commercial performance panel was found to be qualitatively concordant with all other commercially available assays reported on both VITROS systems. The VITROS HTLV I/II Assay* generated similar dilutional performance on both VITROS systems and generated reactive results on samples 4 – 10 X more dilute than were reactive on the AVIOQ HTLV-I/II Microelisa System. Within-lab precision of the assay ranged from 3.7 – 5.7 %CV above the cutoff (>1.0 S/C) and 8.9 – 19.8 %CV below the cutoff on both VITROS systems.

Conclusion:

The VITROS HTLV I/II Assay* has demonstrated excellent sensitivity and specificity, and acceptable precision.

* Under Development

B-107

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

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

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

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

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

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

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

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

B-108

Performance evaluation of the ADVIA Centaur Zika IgM assay*

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

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

Results: ADVIA Centaur ZikaM assay results were reported as reactive for samples with ≥1.00 index and nonreactive for samples with <1.00 index. In 50 Zika PCR-positive serial draw sets (eight draws per individual), all 50 individuals showed reactivity with the ZikaM assay within 2-27 days after the appearance of symptoms, and the majority of these (43/50) were reactive within 14 days post-symptom onset. When the results of all draws from 50 Zika-positive individuals were combined, approximately 85% of the samples were reactive with the ZikaM assay. Evaluation of normal samples from a U.S. population (blood donors and pregnant women) by the ZikaM assay gave a specificity of 94.7% (1418/1497). Specificity in samples collected from Zika-endemic areas was 70.7% (29/41) in individuals with symptoms of Zika and 81.7% (116/142) in asymptomatic individuals. Additionally, cross-reactivity was evaluated using 159 cross-reactive samples, including common flaviviruses (dengue, yellow fever vaccinees, and West Nile).

Conclusions: These results demonstrate good performance of the prototype ADVIA Centaur Zika IgM assay.

*Under development. Not available for sale. The performance characteristics of this device have not been established. Product availability will vary from country to country and will be subject to varying regulatory requirements.

B-109

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

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

Methods: Complete blood count and CPD data measured in the Unicel DxH800 Coulter Cellular Analyzer (Beckman Coulter, USA) were collected retrospectively from 65 patients with confirmed diagnosis of viral infection, acute bacterial infection with (+) sputum culture (n=21), and 106 (viral Ag negative 84 and sputum culture negative 22 cases) controls. Viral diagnosis was made by **combo card test** (CerTest, Biotec, Spain), which is a coloured chromatographic immunoassay for the simultaneous qualitative detection of *RSV* and *Adenovirus* antigens. For bacterial diagnosis; sputum samples were stained by Gram stain, and were inoculated on 5% sheep blood agar, Chocolate Agar, Haemophilus Agar, MacConkey Agar (BioMerieux, France). After overnight incubation, the samples were evaluated. The CPD data include mean (MN) and standard deviation (SD) of volume of lymphocytes and neutrophils, and lymphocyte conductivity. Lymphocyte (LI) index was calculated.

Results: WBC of bacterial culture (+) and viral Ag (+) cases were not different significantly different (7.8, range 6.7-12.3 and 11.5, range 7.5-12.2; p=0.074, respectively). Mean percent neutrophils were significantly higher in bacterial culture (+) cases 75.7 vs 43.9, P<0.001), on the other hand mean percent lymphocytes were higher in viral Ag (+) cases (12.9 vs 40.3, P<0.001). The lymphocyte conductivity (LyC) were significantly higher in the viral infection than the bacterial infection (P=0.004). In ROC analysis, lymphocyte volume SD (LySDV) sensitivity 71% specificity 63.1, LyC sensitivity 71% specificity 59.4 in diagnosing viral infection.

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

B-110

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

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

Methods: The assay was developed as a ready-to-use test containing all the required elements for the amplification of both HHV-6 DNA fragment and human beta-globin gene as internal control. The two sets of primers and probes are combined in a

lyophilized and ready-to-use mix, co-amplified and detected by a Real-Time PCR instrument. In the present study, several samples obtained from San Raffaele Hospital, previously tested with an "in-house" Real-Time PCR test, were investigated. PCR reactions were performed on nucleic acids extracted from plasma, whole blood and cerebrospinal fluid (CSF).

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

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

B-111

Circulating Cytokines and Chemokines Disturbance may Influence HBV DNA Replication in Patients with Chronic HBV Infection

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Background: Host immune plays a critical role in anti-virus responses and liver injury in patients with HBV infection. Regulatory T cells (Treg) and Th17 cells imbalance has been demonstrated to participated in chronic HBV infection (CHB) progression. IL-10 and IL-17 are the characteristic and effector cytokines of Treg and Th17 cells, respectively. Studies on circulating cytokines or chemokines profiles in anti-virus effects or liver inflammatory responses in CHB were rare. Here we aimed to explore the specific effects the inflammatory, anti-inflammatory cytokines as well as chemokines exerted in HBV DNA replication, e antigen seroconversion and the impairment of liver function.

Methods: 159 patients with CHB (including 71 with HBeAg (+) and 88 with HBeAg (-)) and 29 healthy controls (HC) were recruited from outpatients and inpatients in West China Hospital of Sichuan University. Circulating levels of inflammatory cytokines including IL-1β, IL-6, IL-17 and anti-inflammatory IL-10, as well as chemokines comprising IL-8 and IP-10 were measured by Bio-Plex system and Bio-Plex Pro™ human cytokine reagent kits (Bio-Rad, USA). The clinical information including HBV DNA load, HBV serology biomarkers and liver function were recorded.

Results: ① Circulating IL-1β, IL-6, IL-17, IL-10, IL-8 and IP-10 all increased in CHB compared with HC.($P<0.05$). ② In HBeAg positive CHB circulating IL-1β, IL-6, IL-10 and IP-10 were strikingly higher than those in HBeAg negative CHB. ($P<0.05$) And IL-17 significantly decreased, resulting in IL-10/IL-17 ratio rising in HBeAg positive CHB. ③ Compared with HBV DNA>1000 IU/ml group, cytokines and chemokines except for IL-17 were all elevated in HBV DNA≤1000 IU/ml group ($P<0.05$ for IL-1β, IL-6, IL-10, IL-17 and IP-10), as well as IL-10/IL-17 ratio increased. ④The similar differences of circulating cytokines and chemokines levels were observed in ALT group, which showed circulating IL-1β, IL-6, IL-10, IP-10 and IL-10/IL-17 ratio rose distinctly and IL-17 declined in ALT≥40 IU/L group. ⑤ Correlation analysis indicated IL-1β, IL-6, IL-10 and IP-10 were positively related to serum ALT levels (r_s=0.360, 0.381, 0.352 and 0.459, respectively; all P values <0.05), and IL-17 had a negative correlation with ALT level (rs= -0.192,$P<0.05$).

Conclusion: In chronic HBV infection circulating rising IL-1β, IL-6 and IP-10 took part in liver injury and higher IL-17 with lower IL-10 may have a anti-virus role, which was characterized by inhibiting HBV DNA replication and prompting seroconversion to anti-HBe antibodies.

B-112

Rapid screening of urinary tract infection using bacteria mode of UF-5000

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Background: Rapid screening of urinary tract infection (UTI) is advantageous to determine rapidly antibiotic treatment, to decrease inappropriate use of antibiotics, and reduce the unnecessary urine culture. Using a flow cytometry based automated urine analyzer UF-5000 (Sysmex, Kobe, Japan), rapid screening of gram negative and bacteria was made possible. In this study, we compared the results of bacteria mode of UF-5000 and conventional culture and investigated the potential clinical impacts.

Methods: A total of 985 urine samples as collected from 813 consecutive patients who were suspected of having UTI and requested for urine culture were tested. Urine samples were inoculated to culture disk using automated Previ-Isola (bioMérieux, Marcy l'Etoile, France) system and bacterial identification were performed by Vitek 2 (bioMérieux). After inoculation by Previ-Isola, the same tubes of the urine were analyzed by Sysmex UF-5000 within 3 hours after inoculation.

Results: Among total samples, 587 samples (59.6%) presented bacterial growth, with 216 samples presented gram negative bacteria and 321 samples presented gram positive bacteria in urine samples by culture. By receiver operating curve (ROC) analysis the best-cut off of UF-5000 for suspected UTI (with ≥ 1,000 gram-negative bacteria and ≥ 10,000 gram-positive bacteria) was >122.1 bacteria/μL, with the sensitivity and specificity of 72.6% (67.7%-77.1%) and 89.7% (87.0%-92.0%), respectively. The cut-off for screening significant UTI (with ≥ 100,000 gram-negative and/or -positive bacteria) was >118.8 bacteria/μL, with the sensitivity and specificity of 87.4% (82.6%-91.2%) and 85.0% (82.2%-87.5%), respectively. When the bacterial count and white blood cell (WBC) counts of UF-5000 were analyzed in combination, the area under the curve (AUC) was 0.920, and sensitivity was 85.0% and specificity was 86.7% at cut-off of >425.2 bacteria/μL or >155.6 WBC/μL. When the performance of bacterial identification of UF-5000 was evaluated in samples with ≥ 100,000 bacteria, the sensitivity was 86.2% and specificity was 90.3% for reporting the Gram-negative flag as compared with the final bacterial identification results. The reporting time of final results was significantly reduced for UF-5000 as compared with conventional culture ($P < 0.001$).

Conclusion: The use of screening by UF-5000 can give rapid and useful information for determining UTI and identification of gram-negative and positive bacteria. Therefore it can assist prompt and appropriate use of antimicrobial therapy.

B-113

Performance Evaluation of the ADVIA Centaur CMV IgG Assay on the ADVIA Centaur CP System

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Background: Siemens Healthcare (Tarrytown, NY) is currently developing a cytomegalovirus IgG (CMV IgG) 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® CMV IgG assay* is a chemiluminescent magnetic microparticle-based immunoassay that utilizes the NSP-DMAE molecule and runs on the ADVIA Centaur Immunoassay Systems. The objective of this study was to evaluate the positive and negative agreement, precision, and sensitivity of a prototype, automated CMV IgG assay tested on the ADVIA Centaur CP system.

Methods: The fully automated ADVIA Centaur CMV IgG assay is being developed as an indirect sandwich assay for the detection of CMV IgG antibodies in human serum and plasma for use on the ADVIA Centaur CP system. The assay was evaluated for positive and negative agreement (via method comparison), sensitivity, cross-platform alignment, repeatability, and within-lab precision. The positive and negative agreements of the assay were evaluated using a total of 1112 patient samples across two reagents lots. Sensitivity was evaluated using a mix-titer panel obtained from the Center for Disease Control (CDC) containing characterized CMV IgG positive and negative samples. 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 or provided Certificate of Analysis. Discordant samples were tested on the Siemens IMMULITE® 2000 and/or Roche cobas® e 411 CMV IgG assays, when available. Cross platform alignment was evaluated against the ADVIA Centaur XP system, as per CLSI EP9-A2. Precision was evaluated, as per CLSI EP5-A3, by testing four samples with Index values spanning the assay range in two runs per day for 20 days on the ADVIA Centaur CP system for a total of 80 replicates.

Results: Evaluation of the patient samples using the ADVIA Centaur CMV IgG assay indicated that the positive agreement ranged from 99.0% to 99.2% and the negative agreement ranged from 95.3% to 95.8%, when compared to the VIDAS CMV IgG assay. In addition, the ADVIA Centaur CMV IgG assay displayed a total agreement of 99.4% (179/180) to the clinical status of the characterized CMV IgG samples provided by the CDC. Regression analysis comparing sample performance on the ADVIA Centaur XP and CP immunoassay systems, across two lots, yielded a slope ranging from 0.946 to 1.00. The assay demonstrated good precision, with an average

repeatability and within-run %CV of <4.0% and <6.0%, 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 CMV IgG assay on the ADVIA Centaur CP system.

*Under Development. Not available for sale. The performance characteristics of this product have not been established.

B-114

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

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

Materials and Methods: 54 patients with HIV antibody and HBV DNA>10^3IU/ml were defined as the research group, while 56 cases without HIV antibody and with HBV DNA>10^3IU/ml as the control group. HBV DNA was extracted from serum or plasma. Then, nested PCR was performed for the RT and BCP/PC region. After electrophoresis, PCR products were sequenced. The sequencing results were analyzed.

Results: Genotype B was the dominant genotype in HIV/HBV coinfection patients, accounting for 81.48%, while genotype C accounted for 14.82%. The other two cases were C/D recombinant. The total drug resistance rate was 3.70%. A1762T, G1764A and G1896A mutations were the highest mutations in BCP/PC region. A1762/G1764A mutation rate was 35.19%, and G1896A mutation rate was 59.30%. Compared with HBV mono-infection group, there was no significant difference in HBV genotype distribution, drug resistance rate, HBV gene system evolutionary tree and PC mutation rate. And in HIV/HBV coinfection group A1762T/G1764A mutations rate was lower. A1762T, G1764A and G1896A mutation rate of HBeAg negative patients was higher than the HBeAg positive patients for both HIV/HBV coinfected group or mono-HBV infection group.

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

HBV genotype comparison of HIV/HBV coinfection group and HBV mono-infection group

	HIV/HBV coinfection	**HBV mono-infection**
	(n=54)	(n=56)
Genotype B	44(81.48%)	45(80.36%)
Genotype C	8(14.82%)	10(17.86%)
Genotype D	0	1(1.78%)
Recombinant C/D	2(3.70%)	0

B-115

Laboratory Diagnosis of Chikungunya virus: a comparative study of commercial tests for detection of IgM and IgG anti-Chikungunya

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Background: The Chikungunya fever is caused by the Chikungunya virus (CHIKV) with symptoms similar to other arbovirus infection, however polyarthritis/arthralgia are clinical signs that can to last months after fever. This disease has affected millions of people and continues to cause epidemics in many countries. According to World Health Organization it occurs in Africa, Asia and the Indian subcontinent. In recent decades mosquito vectors of Chikungunya have spread to Europe and the Americas. Expansion and endemicity of CHIKV is likely, and large outbreaks of CHIKV infection may continue for the foreseeable future. Clinical laboratories throughout the Americas will need to build and maintain high-volume diagnostic testing capacity and will need validated and reliable commercial CHIKV diagnostic assays to respond to these increased diagnostic testing responsibilities. Commercial kits are available, sometimes with excellent sensitivity and specificity. **Objective**: To compare the performance of two tests for serologic diagnostic of CHIKV on Brazilian samples obtained in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) care routine, using enzyme immunoassay and indirect immunofluorescence. **Methods**: A total of 63 samples were analyzed by Anti-Chikungunya IIFT IgM and IgG kit (Euroimmun AG), and Chikungunya Virus IgM and IgG ELISA kit (Novalisa) according to the manufacturer's instructions. Both tests were approved by a national regulatory agency (ANVISA, Agência Nacional de Vigilância Sanitária). Positive and negative controls were used, which were approved and validated. **Results:** The IgM antibody results showed a concordance of 24 positive samples and 36 negative samples between the tests. The disagreements of 1 negative sample in IIFT and positive in ELISA, and 2 positive samples in IFF and negative in ELISA were evidenced. The negative concordance percentage for IgM was 97.3% and the positive concordance was 92.3%. For IgG antibody, 33 negative and 19 positive samples were observed in both tests, 11 samples were positives in IIFT and negative in ELISA. The percentage of negative agreement was 100% and positive concordance was 63.3%. The Kappa coefficient for IgM was 0,901, with 95% confidence intervals (CI) of 0,654 to 1.0, indicating nearly perfect agreement and for IgG was 0,644, with 95% CI of 0,413 to 0,875, indicating substantial agreement. **Conclusions**: Anti-CHIKV antibodies can be detected in patients shortly after symptom onset, usually after 5 days for IgM and only a few days later for IgG. The CDC published study comparing CHIKV IgM using commercials kits IIF Euroimmun and other ELISA´s and it demonstrate an agreement of >95% to >90%. According to the manufacturers, both techniques show differences in sensitivity (ELISA-Novalisa: > 90%, IIF-EUROIMMUN: 96.2%), which may explain the occurrence of divergences. Despite this, the two kits demonstrate to have equivalent performance. The findings show the importance of evaluating commercial diagnostic kits before using such tools in laboratory routine especially in CHIKV endemic areas and in clinical settings.

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***Mcr1* gene: first detected case in Ceará, Northeastern Brazil**

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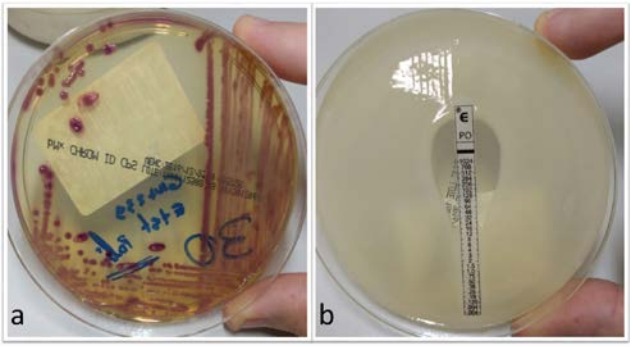
Background: Resistance to polymyxins is already known in Gram negative bacteria due to mutation in chromosomal sites. In 2015, Liu *et al*. described *mcr1* gene as responsible for plasmid-mediated resistance to polymyxins in *Escherichia coli*strains. Since then, some cases have been described in several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

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

Results: A 86-year-old female patient was admitted to the hospital with complaints of general decline and drowsiness for 3 days. She had reported sudden respiratory distress. She was transferred to ICU and it was prescribed Piperacillin-Tazobactam EV. Urineculture and hemoculture were requested. Urine sample was seeded in CPS ID3 agar (BioMerieux ™). After 24 hours at 35ºC, a pink colony was detected (figure 1a). Analysis by Vitek 2 (BioMerieux ™) resulted in *Escherichia coli*identification with resistance pattern to ampicillin, ciprofloxacin and colistin. The MIC found for colistin was 8µg/mL. E-test of polymyxin was performed, which confirmed *in vitro* resistance pattern (figure 1b). The isolate was subjected to real-time PCR and the presence of *mcr1* gene was detected.

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



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Study of the concordance of serological tests for anti-Leishmania antibodies detection

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

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

KIT	IT-LEISH	
R-biopharm	**Negative**	**Positive**
Negative	12	1
Positive	25	17
Novatec	**Negative**	**Positive**
Negative	30	1
Indeterminate	3	2
Positive	4	15
Euroimmun	**Negative**	**Positive**
Negative	33	5
Indeterminate	1	1
Positive	3	12

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MALDI-TOF: an application of mass spectrometry for pathogenic bacteria identification in clinical laboratory.

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Background: Currently, diagnostic methods for microbiological identification of pathogenic bacteria have been carried out classically by methods involving culture and phenotypic/biochemical tests exploring metabolic differences that exist between different species of microorganisms. These methods of identification are extremely powerful methods of pathogen retrieval, however, they are laborious and the results are obtained after a long period of time depending on the pathogen. In some circumstances, such as bacteremia, identification and appropriate treatment are critical. New methods of identification have been developed. Rapid and accurate molecular genetic tests are commercially available for the identification of species, but they are expensive, which is a limiting factor for their implementation in the laboratory routine. Other methods for rapid diagnosis in the etiology of infections are being used, such as mass spectrometry technology. **Objective**: To analyze the process of bacterial identification by Maldi-Tof in clinical laboratory routine. **Methods**: Bacterial strains were isolated from several clinical samples, including peritoneal fluid, bronchial lavage, urine, blood, sputum and oral secretion that were sent to Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) for culture and identification. The culture was carried out in specific media according to each of clinical samples and medical suspicion. Identification of strains was performed in the Vitek System according to the manufacturer's instructions and/or by conventional Pessoa and Silva method. Bacterial identification by mass spectrometry was performed using Vitek MS (BioMérieux) equipment according to the manufacturer's instructions. For calibration of equipment was used a reference strain of *Escherichia coli* ATCC 8739 according to the manufacturer's specifications. The data obtained from the spectrum were transferred from Vitek MS acquisition station to Myla analysis server (BioMérieux) and compared with SARAMIS 4.12 database. **Results**: A total of 214 bacterial strains were analyzed, including 60 enterobacterial strains, 48 *Staphylococcus* strains, 56 catalase negative cocci bacteria, 50 non-fermenting and fastidious Gram negative rods strains. The concordant results between the two identification techniques were summarize as follows: 90% (54/60) of enterobacterial strains; 92% (44/48) of *Staphylococcus* strains; 98% (55/56) of catalase negative cocci bacteria; 98% (49/50) of non-fermenting and fastidious microorganisms. **Conclusion**: The Maldi-Tof system showed an identification efficiency and workflow robustness compared to routine phenotypic identification in laboratory. This technique has revolutionized microbiological clinical diagnosis because it is a faster workflow, accurate and low cost specimen identification method. The use of mass spectrometry in clinical and research laboratories in European countries is already a reality. In Brazil, the first mass spectrometer with this application was installed in 2010 and this tool is already being used by some research groups and clinical laboratories. Further investigations are need to directly identify bacteria from clinical samples and detect drug resistance among bacteria. These studies are a challenge for clinic microbiologists and might guide patient decision-making regarding bacterial infectious diseases in the near future.

B-119

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

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

Methods: HAVT and HAVM are competitive and non-competitive immunoassays, respectively, performed with the fully automated VITROS 3600 Immunodiagnostic

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

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

Assay	Expected qualitative result	Mean S/CO ratio observed in serum aliquots with the following biotin concentrations in ng/mL					
		0	10	50	100	500	1,000
HAVT	Negative	1.74	1.75	1.60	0.980[a]	NT	NT
	Negative	1.25	1.17	0.990[a,b]	0.660[a,b]	0.290[a,b]	0.120[a,b]
	Reactive	0.550	0.540	0.470[a]	0.340[a]	0.050[a]	0.035[a]
	Reactive	0.030	0.030	0.025[a]	0.020[a]	NT	NT
HAVM	Negative	0.010	0.010	0.010	0.010	0.010	0.010
	Reactive	1.67	1.67	1.63	1.60	1.64	1.62
	Reactive	6.86	6.85	6.85	6.77	6.65	6.69

[a] >14% difference from corresponding ratio at 0 ng/mL;
[b] Change in qualitative interpretive result;
NT, not tested.

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

B-120

Evaluation of serologic laboratorial test in brazilian patients infected by Zika virus and exposed to Dengue virus and Chikungunya virus.

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Background: Zika virus (ZIKV) is virus whose the newest pandemic is alarming, although, with the paucity of literature the exact details of the disease are not clear. The diagnosis of ZIKA infections can be performed on clinical-epidemiological and laboratorial bases. Overall, your laboratorial diagnosis relies on the same usual strategies used for other arboviruses, with viral genome detection by RT-PCR tests on acute-phase samples and serology (ELISA and immunofluorescence). Usually, the choice of the laboratorial approach used will depend on the goal of the analysis, laboratory infrastructure, technical expertise and sampling availability.The diagnosis of ZIKV infection performed by serological tests can detect specific IgM / IgG antibodies against ZIKV after 5 to 6 days of the onset of symptoms, with increased titers within 2 weeks. **Objective:** To evaluate the performance of commercial Euroimmun ZIKV ELISA test (Euroimmun, Lübeck, Germany) using panels from Brazilian patients exposed to Dengue (DENV) and Chikungunya (CHIKV) infection. **Method:** Samples were obtained in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) routine, with a high potential of causing cross-reactions in serological flavivirus assays. The selection of samples was based on six panels for IgM analysis (n=105) and four panels for IgG analysis (n=65). For IgM assay the panels were as follows: Panel I: 20 DENV IgM-positive samples; Panel II: 15 DENV IgM and IgG-negative samples; Panel III: 15 CHIKV IgM-positive samples; Panel IV: 15 CHIKV IgM and IgG-negative samples; Panel V: 20 Epstein Barr virus IgM-positive samples; Panel VI: 20 Rheumatoid Factor (RF) IgM-positive samples (Concentration: 153.5 to 1350.9 IU/mL). For IgG assay the panels were as follows: Panel I: 20 DENV IgG-positive; Panel II: 15 DENV IgM and IgG-negative samples; Panel III: 15 CHIKV IgG-positive samples; Panel IV: 15 CHIKV IgM and IgG-negative samples. All assays were performed according to the instructions of the manufacturer. **Results**: The ZIKV IgM ELISA was negative in all simultaneously DENV and CHIK negative samples, CHIKV positive, EBV positive and RF positive samples. Of DENV positive samples 10% were ZIKV IgM positive (2/20). For IgG ELISA, 20% of DENV positive samples were ZIKV IgG positive (4/20). All simultaneously DENV IgM and IgG negative samples were ZIKV IgG negative. Of CHIKV IgG positive samples 60% were ZIKV IgG positive (9/15). In addition, 33% of simultaneously CHIKV IgM and IgG negative samples were ZIKV IgG positive (5/15). **Conclusion**: Positive samples for the dengue virus may also be positive for ZIKV. No cross-reactivity of IgM ZIKV with Chikungunya virus has been observed. The IgG positive samples for dengue and Chikungunya viruses may be positive for ZIKV. Despite this it is not established if this situation corresponds to a cross reaction of the test or simply histories of coinfection in different periods in the same individual in endemic areas. Its positivity should be evaluated in the context of the other conditions, such as Epstein Barr virus infections, and in malaria infection. Further studies will be necessary to determine the accuracy of this test and other current assays in a larger set of well-defined samples.

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

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

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

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

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

assessed at three sites using three reagent lots with a 14 member panel. Antigen sensitivity was determined by testing serial dilutions of the NIBSC and the AFSSAPS HIV-1 p24 Ag standards across two reagent lots.

Results: The specificity of the VITROS HIV Combo Assay* for the low risk population was 99.59% (6365/6391) [95% exact CI (99.40-99.73%)]. Samples confirmed as HIV positive by supplemental testing were removed from the analysis (n=44). The sensitivity for HIV-1 and HIV-2 antibody positive samples was 99.94% (1763/1764) [exact 95% CI (99.68-100.00%)]. All 413 HIV-1 group M and O antibody positive subtypes and 50 of 52 HIV-1 group M antigen genotypes were reactive with the VITROS HIV Combo assay*. For seroconversion panels the VITROS HIV Combo assay* was reactive at the same panel member as the commercially available Ag/Ab assay for 27 of the 34 panels, was reactive one panel member earlier for 6 panels, and was reactive two panel members later for 1 panel. For the reproducibility study the observed precision for the 12 panel members positioned near the assay cutoff ranged from 9.1 to 17.8 %CV. The overall sensitivity of the VITROS HIV Combo test for the NIBSC HIV-1 p24 Antigen Standard (90/636) was ≤0.48 IU/mL and for the AFSSAPS HIV-1 p24 Antigen Standard was ≤13.1 pg/mL.

Conclusion: The VITROS HIV Combo Assay* demonstrates acceptable clinical and analytical performance in the simultaneous detection of antibodies to HIV-1 (group M and O), HIV-2 and HIV p24 antigen.

*Not approved for use in the US.

B-123

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

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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 throughout countries of the Americas and more recently Pacific island nations, as well as its link to neurological birth defects. In response to this outbreak, several PCR-based Zika assays have been developed and approved under FDA's Emergency Use Authorization. Clinical laboratories as well as test developers need to evaluate assays across the entire reportable range for sensitivity and linearity. SeraCare has developed the Accuspan Zika Linearity Panel to meet this need. The panel uses recombinant Sindbis virus technology; therefore, it is non-infectious but is a whole process reference material that must undergo the entire extraction procedure. This study reports the formulation of the panel as well as initial testing.

Methods: The Zika virus is a positive sense RNA virus whose genome is approximately 10.7 Kb. The Zika genome was divided into four (4) segments and each segment was used to generate a recombinant virus using Sindbis vector system. The recombinant viruses were heat inactivated and purified. The strategy of dividing the Zika genome into four different recombinant viruses such that each recombinant virus is not functional assures the safety of the reference material and the heat inactivation serves as an additional safety precaution. Digital PCR assays, specific for each recombinant virus construct (Envelop region, NS2/NS3 region, NS4 region and NS5 region) were designed and used for quantitation of each viral stock. Based on digital PCR quantitation, the viral stocks were mixed together in equimolar ratios such that the combined bulk contained sequences of the entire Zika genome. The combined bulk was then serially diluted in defibrinated human plasma to titers of 1.0E+06, 1.0E+05, 1.0E+04, 1.0E+03, 1.0E+02, and 1.0E+01 copies/mL. Titers of each member were verified by digital PCR testing. A negative member (containing the plasma diluent) is also included in the panel.

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

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

B-124

Biomarkers of inflammation in cerebrospinal fluid and serum to differentiate between bacterial and viral meningitis

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Background: The analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture is used as test for diagnosis of meningitis. There are a great number of works published on biomarkers of inflammation for the diagnosis of meningitis, but there are discrepancies on their utility clinical, not being clear if its determination in CSF presents a greater accuracy that in serum. The aim of this study was to determine the accuracy of the albumin, lactate dehydrogenase (LDH), c-reactive protein (CRP), procalcitonin (PCT) and ferritin in CSF and serum to differentiate between bacterial and viral meningitis.

Method: We study patients with clinical suspicion of meningitis. Two types of samples were analyzed: CSF by lumbar puncture and peripheral venous blood obtained by venipuncture. Albumin, LDH and CRP were quantified in the autoanalyzer Dimension EXL (Siemens®), ferritin in the Modular E-170 (Roche Diagnostic®) and the PCT in the Kriptor (Thermo Scientific®). The diagnosis of the patient was obtained from their medical history. Patients diagnosed with bacterial and viral meningitis were selected. The accuracy for diagnosis was determined using receiver operating characteristic (ROC) techniques by analysing the area under the ROC curve (AUC).

Results: We studied 156 patients with age between 1 and 86 years (median = 54 years), 64 women and 92 men. 18 were diagnosed of bacterial meningitis and 12 of viral meningitis. Biomarkers analyzed in CSF and/or serum showed higher concentrations in patients with bacterial meningitis which in the viral. The AUC to differentiate bacterial from viral meningitis with biomarkers analyzed in CSF, serum and with its ratio (CSF/serum) are shown in the following table:

	CSF	Serum	CSF/Serum
ALBUMIN	0.750 (p=0.0088)	p>0.05	p>0.05
LDH	0.704 (p=0.0327)	p>0.05	0.796 (p=0.0003)
PCR	0.935 (p<0.0001)	0.926 (p<0.0001)	0.806 (p=0.0001)
PCT	0.694 (p=0.0440)	0.963 (p<0.0001)	0.870 (p<0.0001)
FERRITIN	p>0.05	0.852 (p<0.0001)	0.963 (p<0.0001)

Conclusions: Albumina is useful to differentiate viral from bacterial meningitis when is it quantified in CSF; LDH presents greater accuracy when using the CSF/serum ratio; PCR when determined in CSF; the PCT when they are quantified in serum; and ferritin when using ratio CSF/serum.

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A Rapid Syphilis Test for Qualitative Measurement of Treponemal Antibody

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Background: Syphilis is a sexually transmitted disease caused by bacteria called Treponema pallidum (TP). Most people with syphilis tend to be unaware of their infection and they can transmit the infection to their sexual contact or in the case of pregnant woman, to her unborn baby. If left untreated, syphilis can cause serious consequence such as stillbirth. According to WHO, syphilis kills more than one million babies a year worldwide, and 12 million new cases of syphilis occur every year. In addition, as a cause of genital ulcer disease, syphilis has been associated with an increased risk of HIV infection. Early diagnosis is important because timely treatment of syphilis significantly prevents its serious consequence and the spread of the disease. An easy-to-use rapid test for the detection of Treponemal antibody can facilitate an early diagnosis of a syphilis. The objective of this study is to evaluate the performance of a new syphilis rapid test. **Principle:** The ADEXUS-Dx Syphilis Rapid Test ("**Syphilis Test**") is a solid phase immunochromatographic assay. The Syphilis Test uses a sandwich format to detect the presence of Treponemal antibody in blood, plasma, and serum samples. The appearance of a purplish-red band in the test window indicates that the sample contains Treponemal antibody. The Syphilis Test has a unique feature of finger-stick, capillary whole blood sampling needing only 35 µl blood. No additional buffer is needed. The result is ready in 15 minutes. **Performance:** 100 known Treponemal antibody positive samples were tested by the ADEXUS-Dx Syphilis Rapid Test and 99 tested positive. The calculated clinical sensitivity is 99%. Within these 100 positive patient samples, 51 are serum, 26 are EDTA plasma, 11 are heparin plasma, and 12 are citrate plasma. The expected results were obtained for both serum and plasma samples, indicating the test was unaffected

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

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

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

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

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

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

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

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The FSC PathOne™ Analyzer: A Novel Platform for the Rapid Diagnosis of *Pseudomonas aeruginosa* in Urinary Tract Infection

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Objective: We developed and validated an assay for the rapid diagnosis of *P. aeruginosa* in urine on a novel detection platform, the PathOne™Analyzer from Fundamental Solutions Corporation. **Relevance:** Urinary tract infections are a serious health problem and the second most commonly diagnosed infection of any organ system in the United States. Catheterization is a risk factor for developing UTIs, and catheter-associated UTIs (CAUTI) account for more than 1 million cases in hospitals and nursing homes annually. *P. aeruginosa* is an important pathogen for CAUTI, representing approximately 10% of all cases, of which approximately one third are fluoroquinolone-resistant. *P. aeruginosa* is one of the ESKAPE pathogens and is of particular concern due to its ability to form biofilms on the surface of urinary catheters and its resistance to many antibiotics. Therefore, rapid and accurate diagnosis is needed to provide patients with the correct antibiotics and improve patient outcome. **Methods:** The PathOne analyzer is a novel, rapid, portable diagnostic device and cartridge system that was designed for the true real-time detection of pathogens from sample to result in less than 5 minutes with no enrichment. The technology is a live cell-based biosensor which rapidly detects the presence of an offending target pathogen through a proprietary, light-emitting reaction. In this study, we developed an assay to detect *P. aeruginosa* in contrived urine samples. Assay limit of detection, specificity, range and linearity was determined. Results were obtained using urine pooled from 10 donors and confirmed using contrived samples from individual donors. Contrived samples were generated to mimic bacterial load found in clinical samples. **Validation:** We have in our collection 50 isolates of *P. aeruginosa* with well-characterized resistance profiles against 15 antibiotics representing the major drug classes. These isolates were used to generate contrived urine samples to mimic bacteriuria found in the clinic. Data were analyzed to determine within-run precision, total precision and accuracy. **Results and Conclusions:** We have developed a novel, rapid diagnostic test for *P. aeruginosa* in urine with acceptable sensitivity, specificity, precision and accuracy.

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A New One-Step Direct-Sampling Hepatitis B Surface Antigen Rapid Test

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

(78 positives and 110 negatives measured by Abbott EIA) were tested . The overall relative sensitivity and specificity for the detection of HBsAg were 96% and 94% respectively. Fresh normal capillary blood test showed negative results with good plasma separation. **Conclusion:** The ADEXUS-Dx HBsAg Test is a true one-step rapid test suitable for capillary blood testing. It demonstrated good sensitivity and specificity. It is suitable for use in the detection of HBV infection at the point-of-care settings and for self-testing.

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Identifying Significant Association between Host Factors and Improvement of QF TB-Gold Testing with Decreased Incubation Periods

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Background:

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

Methods:

In January of 2016, our institution implemented a one hour incubation period for Quantiferon-TB Gold samples, replacing the previous 16 hour incubation period maximum. In this study, we retrospectively identified 112 patients who received QF testing in the 5 months prior to implementation and 141 patients who received QF testing in the 12 months after implementation of 1hr incubation. Seven clinical risk factors associated with indeterminate results were evaluated.

Results:

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

Conclusion:

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

Table 1:

	16-Hour incubation Period	1-Hour incubation period	P Value
Hypoalbunemia	45.7%	65.2%	0.040
Cirrhosis	21.7%	40.9%	0.034
Malignancy	13.0%	13.6%	0.928
Autoimmune disease	50.0%	47.0%	0.752
Chronic Inflammatory disease	50.0%	43.9%	0.527
Hepatitis B	0.0%	1.5%	0.402
Hepatitis C	10.9%	15.2%	0.753