Wednesday, July 30, 2014

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

Infectious Disease

B-035

Comparative Study of Granada and ChromoID StreptoB media for identification of Group B Streptococcus

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Background: BIOMÉRIEUX StreptoB Granada and ChromoID media are used to identify group B Streptococcus. Microbiological screening of these bacteria is crucial since it can lead to neonatal septicaemia in pregnant women. Generally, it appears in the first 24 hours of life and often results in fulminant septicaemia, meningitis, or pneumonia associated with high morbidity and mortality. The most effective strategy to reduce the incidence of GBS in newborns is a pre-natal tracking of all pregnant women between 35-37 weeks of gestation time. This is done using culture methods to determine the necessary intrapartum antibiotic prophylaxis. The objective of this study was to compare the effectiveness of the Granada medium at identifying Group B Streptococcus in samples of vaginal and perianal openings.

Methods: A 100 samples of vaginal and perianal openings of women with 35-37 weeks of gestation were collected and transported in Stuart and AIMES transport at room temperature. The procedure was carried according to the package insert. After 24 hours of incubation at 35 °C, macroscopic analyses in the broth were carried out to detect any orange coloring, and if so, samples were positive for group B Streptococcus. Those samples were seeded in the ChromoID StreptoB biphasic Granada broth to compare both tests and verify which one provides more effective and precise results. Inoculated plates were incubated in micro-aerobic culture systems at 35 °C for a duration of 12 hours.

Results: More than half of the 100 samples analyzed showed incompatibilities between the results, especially the Granada ID medium presenting an average of less than 45% positivity when comparing to the ChromoID StreptoB.

Conclusion: In the presented data, three types of tones were considered: strong, intermediate, and weak. A weak-colored orange still resulted in a clinical evaluation of the sample and the patient. If the result returned positive, the patient was returned to isolation and identified as positive for group B Streptococcus. The Granada ID test was shown unreliable if done alone. In order to achieve better accuracy, this test should be done with another identification technique, such as the camp-test or even Chromo ID (STRB). The latter presents excellent specificity and is relatively easy to execute. Its only limitation is that it takes12 hours longer than Granada ID to achieve results.

B-037

Automated Sample-to-Results Analysis of Clinical Specimens for Sexually-Transmitted Infections

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Background: The global burden of sexually-transmitted diseases (STDs) is considerable with an estimated 340 million new cases occurring each year. Although many of these new cases could potentially be effectively cured with modern antibiotic therapy, the early stages of the infections can often go unnoticed. Females are disproportionately affected, in whom untreated STDs can proceed to disabling pelvic inflammatory disease which, in turn, can lead to infertility, infant mortality and infant blindness. Complications in untreated males, although rarer, can proceed to urethritis, epididymitis, as well as infertility. In order to streamline testing, we have developed a fully automated molecular detection system to simultaneously detect N. gonorrhoeae (NG), C. trachomatis (CT) and T. vaginalis (TV) in an unattended manner from a variety of specimens.

Methods: An injection molded disposable CARD (Chemistry & Reagent Device) was developed that, when inserted into the EncompassMDx™ workstation, can automatically lyse cells, extract and purify DNA, multiplex PCR amplify rRNA genomic targets in NG and TV and cryptic plasmid DNA of CT. In order to confirm that all steps of the assay were performed correctly by the system, three separate chimeric plasmids were designed that harbor unique DNA sequences that can be amplified by the same primer pair sets designed to amplify the individual targets of CT, NG, and TV. Hybridization of the control amplicons can be detected and distinguished from hybridization of target amplicons on the integrated DNA array.

Results: Several different clinical reference laboratories provided us with approximately 100 diverse specimens (vaginal swabs, endocervical swabs, and urine specimens), previously tested using FDA-cleared devices in their facilities. Evaluation of the same samples with the Rheonix CT/NG/TV CARD assay yielded similar results. Moreover, since the FDA-cleared test was only able to test for the presence of CT and/or NG, a number of samples were also found to be co-infected with TV. In addition, the use of the chimeric plasmid controls yielded positive signals on all runs, thus confirming that each step of the fully automated assay was properly conducted by the unattended system. Furthermore, to confirm that the DNA arrays were properly orientated in the CARD, spotting controls that display signals were placed on the DNA array in a defined pattern. In order to assure that the proper organisms were detected, the imaging software was designed to only accept results that displayed the proper spotting control orientation, thus confirming that the microorganism(s) detected were correctly scored.

Conclusion: The ability to analyze specimens in a fully automated, sample in_result out format, will allow detection and identification of three sexually transmitted infections to be performed by individuals of varying skill level. The automatic performance in an unattended manner of all sample preparation, DNA purification, amplification, end-point detection, analysis and readout functions makes the platform suitable for central lab, point-of-care, as well as non-traditional healthcare settings. Clinical studies intended to gain FDA clearance are expected to be undertaken in 2014

B-038

A Novel Enzyme-Linked Immunosorbent Assay for the Detection of Nontreponemal Antibodies in the Sera of Patients with Syphilis.

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Background: We describe an enzyme-link immunosorbent assay (EIA) for the detection of nontreponemal antibodies. This assay is ideal for the automation of high throughput screening of sera

Methods: The nontreponemal (cardiolipin) antigen was chemically modified and it was attached covalently to amine functionalized micro titer plates.

Results: A total of 1,006 banked serum samples were evaluated and the results compared to a quantitative rapid plasma regain (RPR) test. The accumulative reactive concordance of the nontreponemal EIA was 93.3% when the RPR titer of the sera was 1:1, 96.2% at 1:2, 98.5% at 1:4, 99.3% at 1:8 and 100% at ≥ 1:16. The nonreactive concordance was 100%. Also 50 samples with known stages of syphilis and 158 from diseases other than syphilis were included.

Conclusion: These results indicate that the nontreponemal EIA test can be used for the screening of large volume of samples using the traditional syphilis testing algorithm of screening with a nontreponemal test and confirming the results with a treponemal test.

B-039

Evaluation of the Alere Determine™ HIV-1/2 Ag/Ab Combo Kit for the Rapid Determination of Antibody/Antigen Status in STAT Specimens in a Busy Metropolitan Hospital Setting

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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 Alere Clearview® STAT-PAK® point-of-care device for HIV-1/2 Antibody STAT testing (needle stick; Labor & Delivery cases). The Abbott ARCHITECT® 4G immunoassay (IA) with reflex to BioRad Multispot HIV-1/HIV-2 Rapid Test kit is used for routine testing. When the Multispot does not "confirm" a reactive ARCHITECT result, serum is referred for further HIV-1 RNA qualitative testing.

OBJECTIVE: The Alere 4G Determine HIV-1/2 Ag/Ab Combo device was recently approved by the FDA. The ARCHITECT reports a signal-to-cutoff ratio for combined HIV-1/2 antibodies and p24 antigen (HIV-1), whereas the Determine distinguishes the HIV-1/2 antibody result from the HIV-1 p24 antigen result. We were, therefore,

interested in evaluating the Determine as a replacement for the current Clearview method for STAT testing.

STUDY DESIGN: 111 serum samples from 106 patients, previously tested for HIV status, were re-tested by the ARCHITECT/Multispot and then by the Clearview and Determine rapid tests. Reactive samples had been stored frozen for up to 18 months, however, there was no significant difference between original and repeat results. Most non-reactive samples had been refrigerated for up to 5 days. ARCHITECT algorithm results and chart review were used for definitive HIV diagnosis.

RESULTS: Two samples from a HIV-2 antibody positive patient were reactive by all methods. Sensitivity and specificity for HIV-1 testing are shown:

Test Systems	Sensitivity (%)	Specificity (%)
Abbott ARCHITECT 4th Generation IA (n=109)	100	77
Alere Determine HIV-1/2 Ag/Ab Combo kit (n=109)		
HIV-1 Antibody	90	93
p24 Antigen	17	98
p24 and/or Antibody	98	92
Alere Clearview HIV-1/2 STAT-PAK (n=108)	83	100
BioRad Multispot HIV-1/HIV-2 Rapid Test (n=109)	83	100

CONCLUSIONS: The Alere Determine HIV-1/2 Ag/Ab Combo kit had an overall sensitivity of 98% for detection of HIV-1 p24 antigen and/or antibody while the Clearview, which does not detect HIV-1 p24 antigen, had a sensitivity of 83%. Therefore, the Determine Combo would be an improvement over the current method for the detection of HIV-positive patients for STAT testing.

B-040

Evaluation of Analytical Sensitivity and Workflow of the VERSANT Hepatitis C Virus Genotype 2.0 Assay (LiPA)

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Background: The VERSANT® HCV Genotype 2.0 Assay (LiPA) is a reverse hybridization line probe assay that uses sequence information from both the 5' untranslated region (UTR) and the core region to accurately distinguish between HCV genotypes 1 to 6 and subtypes 1a and 1b. Prior studies have shown that the assay can genotype 96% of HCV samples with 99.4% accuracy.(1) Assay steps have been automated to improve efficiency and decreased time to results. This study evaluates assay workflow and analytical sensitivity.

Methods: The VERSANT HCV Genotype 2.0 Assay (LiPA) is run in three steps: extraction, amplification, and genotyping. Viral RNA is extracted from plasma or serum using the VERSANT Sample Preparation 1.0 Reagents. The 5' UTR and core regions of HCV are amplified using RT-PCR and the VERSANT HCV Amplification 2.0 Kit (LiPA). Biotinylated amplicons are hybridized to immobilized oligonucleotide probes on nitrocellulose strips and visualized using reagents in the VERSANT HCV Genotype 2.0 Assay (LiPA) Kit. Processed strips are interpreted using the optional LiPA Scan software to yield the HCV genotype. Assay intermediates from each step can either be processed immediately or stored at defined conditions. Analytical sensitivity was evaluated using one specimen for each genotype (1a, 1b, 2, 3, 4, 5, and 6) diluted separately in serum and plasma. Dilution series were prepared at concentrations ranging from 50 to 2000 IU/mL, and each target concentration was tested in multiple replicates and runs with multiple reagent lots on different days. These data are analyzed using a regression method with probit link function.

Results: Automation of extraction and strip processing allows for simultaneous processing of 94 samples. Extraction and loading of the PCR plate have been optimized with the VERSANT kPCR Sample Preparation module, a fully automated instrument for isolation and purification of nucleic acids using magnetic-bead extraction technology. Genotyping has been optimized on the automated Auto LiPA 48 Genotyping Instrument (Strip Processor), which can process up to 46 samples and 2 controls per run. Assay times for 94 samples are 3.5 hours for extraction, 4 hours for amplification, and 4 hours for genotyping (with two Auto LiPA 48 processors), which includes a hands-on time of 2 hours. Initial assessments of analytical sensitivity, measured as the limit of detection for individual genotypes/subtypes, was less than or equal to 500 IU/mL. Further assessments are underway to confirm the analytical sensitivity.

Conclusion: The VERSANT HCV Genotype 2.0 Assay (LiPA) is a sensitive and reliable HCV genotyping assay. Automation of the VERSANT HCV Genotype 2.0 Assay (LiPA) workflow results in higher throughput, improved efficiency, and a decreased time to results.

References: 1.Verbeeck J, Stanley MJ, Shieh J, Celis L, Huyck, E, Wollants E, Morimoto J, Farrior A, Sablon E, Jankowski-Hennig M, Schaper C, Johnson P, Ranst MV, Brussel MV. J Clin Microbiol. 2008;1901.

VERSANT HCV Genotype 2.0 Assay (LiPA) is CE-marked in Europe and for research use only (RUO) in the U.S.

B-041

Quantitation of IFN-γ and IFN-γ induced chemokine mRNA expression levels in active pulmonary tuberculosis patients for effective monitoring of anti-TB therapy

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Background: Tuberculosis (TB) that is mainly caused by *Mycobacterium tuberculosis* (MTB) remains a major global health problem, with approximately 9 million new TB cases annually, leading to 1.4 million in 2011. The results of several previous studies showed numerous cytokines have been implicated in the pathogenesis, diagnosis and control of MTB infection. Especially, interferon gamma (IFN-γ)-specific response to the MTB specific antigens can be used as biomarker for differentiation of active TB and latent tuberculosis infection (LTBI). However, there is an urgent need of prognosis markers for tuberculosis (TB) to determine the response to therapy and improve treatment strategies. **Methods:** In this study, the messenger RNA (mRNA) expression levels of IFN-γ and IFN-γ induced chemokines (MIG, IP-10 and I-TAC) were quantitatively measured by using real-time RT-PCR. For effective anti-TB therapy monitoring, blood sampling, MTB specific Ag stimulation, and molecular assay were performed with a total of 32 active PTB patients at the time of diagnosis (before therapy) and after therapy completion (6 months later). **Results:** The target genes (IFN-γ, MIG, IP-10 and I-TAC) mRNA expression levels were significantly changed and showed a statistical significance at the time of therapy completion from the initial diagnosis active PTB (IFN-γ; p=0.0387, MIG; p<0.001, IP-10; p<0.001, I-TAC; p<0.001). **Conclusion:** In conclusion, data show that the analysis of IFN-γ and IFN-γ induced chemokine mRNA expression levels after MTB specific Ag stimulation could provide useful information during the anti-TB therapy of active PTB patients group.

B-043

Proficiency Test for Laboratory Identification of *Corynebacterium diphtheriae* in Health Care System at Lower Northern Thailand

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Background: Diphtheria is an acute, communicable infectious disease of upper respiratory tract caused by toxigenic lysogenized strains of *Corynebacterium diphtheriae. The incident* in Thailand often found in June to February. The immunized vaccination can only reduce the violation of diphtheria, but cannot be used for extremely protection from re-emerging of diphtheria. Therefore, the rapidly accurate identification of *Corynebacterium diphtheriae* for diagnosis is important for prevention and control. Rehabilitation, practical training laboratories were not enough for confidential results. The proficiency test (PT) program was an assurance implement to use for evaluation of efficiency and quality of laboratory.

Methods: Eighteen hospitals in nine provinces of Lower Northern from Thailand were enrolled in the PT program. The accuracy Identification of *Corynebacterium diphtheriae* was evaluated by statistically from three different PT samples which were homogeneous and stable and were prepared from the same matrix of throat swab in Amies transport medium .

Results: One hundred percentages (16/16) of PT results have been reported and returned to PT provider within the period prescribed. Accuracy identification of the three PT samples was calculated and revealed for 87.5 % (14/16). However, there was 13.5% (2/16) represented inaccuracy with uncorrected results. The satisfaction of PT program was rated for 90.0 %.

Conclusion: The PT program for quality laboratory identification of *Corynebacterium diphtheria*e in health care system at Lower Northern from Thailand was the effectively implement for the sixteen- laboratory participants .

B-044

Screening on Sexually Transmitted Infections among pregnant women

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Background:STI constitute a serious threat to reproductive health of the person in the form of possible complications or increase in risk of transmission of HIV.50 - 60% of STI in female organism proceed without symptoms, causing serious consequences such as pelvic inflammatory disease, tubal infertility,ectopic pregnancy. Children and pregnant women are especially vulnerable concerning STI.The low health index of women of reproductive age and the complicated pregnancy period because of transferred STI, lead to the birth of newborns with a low and very low mass of a body, and is at the bottom of 15% of cases of early neonatal mortality. Prenatal screening of pregnant women can prevent development of the listed above complications. In Kazakhstan protection of motherhood and the childhood is one of the priority directions of a strategic course of development of health care. According to the resolution of the president of Kazakhstan Republic "About Motherhood and Childhood Protection" from 12.12.2003r, one of additional expenses for rendering the state volume of free medical care is inspection of pregnant women on pre-natal infections, congenital anomalies and STI.

Methods:Within the program - screening in clinic laboratory of Skin - venereal specialized health center (East - Kazakhstan region) since 2004 to 2012 examination of pregnant women on DNA STI identification - Chl.trachomatis, Ureaplasma spp., Mycoplasma hominis, Trichomonas vag., Gardnerella vag. Patient material: the fence of a material for researches of pregnant women was spent from an urethra and vagina by disposable urogenital probe in special transport medium. Used method nucleic acid amplification tests (NAATs) - Polymerase chain reaction (PCR),"AmplySens" (Russia).

Results:From 2004 to 2012 were surveyed 19116 pregnant women. Screening was carried out during different terms of pregnancy. The majority of women passed screening on early terms. The percentage of detect ability on 5 infections has been analyzed. On the first place on detect ability is conditionally pathogenic flora - Ureaplasma spp. - 41,8 %, Gardnerella vaginalis - 32%, Mycoplasma hominis - 17%. Among pathogenic flora on first place on detect - Chl.trachomatis - 10% of and Trichomonas vag. - 4%. The share of mono-infection has made - 38%, mixed - infection - 62%. The highest percentage of occurrence of different commensal belongs to the association - Ureaplasma spp - Gardnerella vag. - 45%, Ureaplasma spp. - Mycoplasma hom. - Gardnerella vag. - 17%, Chl.trachomatis - Ureplasma spp. - 8%, Chl.trachomatis - Trichomonas vag. - 3%.

Conclusion:Factors which strengthen potential pathogenicity are: violation of the immunological reactivity. Change of hormonal background. All these factors promote development of diseases of a small pelvis with massive colonization of the urogenital tract. Prenatal screening of pregnant women can prevent the development of premature placental abruption, uterine inertia fetal hypoxia, placentation abnormalities of the fetus in STI.

B-045

Traditional clinical laboratory tests for Dengue fever diagnosis in a children´s hospital, São Paulo, Brazil.

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Background Dengue fever (DF) and dengue hemorrhagic fever (DHF) the more severe form of dengue illness. Dengue viruses are transmitted though the bite of an infected mosquito *Aedes aegypti* is the primary mosquito vector, however other species can also be vectors of Dengue virus. Illness caused by dengue viruses can range from nonspecific febrile illness, as the most DF cases, to more severe illness with bleeding, thrombocytopenia, and plasma leakage in cases of DHF. Dengue incidence and prevalence are rising in endemic areas of the tropical and subtropical regions. Dengue infections occur in more than 100 countries in the Asia-Pacific region, the Americas, the Middle East, and Africa, and cases of infection continue to rise worldwide.3-5Approximately 50 million infections are estimated to occur each year.3 Dengue incidence rates are increasing mainly in tropical and subtropical regions of the world, and in the Americas, a dramatic increase of cases has been reported during the last decades.

Methods: We establish a classification of the results according with the clinical laboratory findings by comparing the results of the tourniquet test and the platelets of samples from all children attended on a Children´s Hospital in São Paulo, SP, Brazil.

Results: 249 tests were analyzed from January to December 2013, March, April and May had the highest number of tests, we divided the classification of the results in four groups, group A negative tourniquet test with normal platelets count, group B negative tourniquet test with low platelets count (thrombocytopenia), group C positive tourniquet test with normal platelets count and group D positive tourniquet test with low platelets count (thrombocytopenia). Group A had the highest combination 201 tests, negative tourniquet test with normal platelets, this result shows that for all suspected cases we have a low incidence of positivity, group B 24 combination (negative tourniquet test with low platelets count), group C 16 combinations (positive tourniquet test with normal platelets count) and group D 4 combination (positive tourniquet test with low platelets count (thrombocytopenia).

Conclusion: Traditional tests for diagnose Dengue Fever is not the gold standard, we expected to have a high number of combination os group D (positive tourniquet test with low platelets count (thrombocytopenia) which shows more evidence to diagnose DF however group A showed us that both tests are still very much solicited by clinical and is not helpful for the diagnose. This way laboratory did not contribute to the DF diagnose though It´s available other tests that can substitute and be helpful such as serology (had number of tests during this period), rapid test and also PCR. There are currently more advanced tests for the diagnosis of dengue fever of which confer higher sensitivity and specificity and contrite to more accurately diagnosing the disease.

B-046

A Multiplex Real-time PCR Assay for the Rapid Detection of the *mec*A Gene with Staphylococci Directly from Positive Blood Cultures

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Background : Sepsis causes increasing morbidity and mortality, particularly in elderly, immunocompromised patients, and it represents one of the greatest challenges in intensive care medicine. Staphylococci are the most commonly isolated organisms accounting for almost 50% of sepsis. In addition, Methicillin-resistant *Staphylococcus aureus* (MRSA) is the most prevalent cause of sepsis and is recognized as a major nosocomial pathogen. This study aimed to evaluate a newly designed multiplex real-time PCR assay capable of the simultaneous detection of *mec*A, *S. aureus,* and coagulase-negative staphylococci (CoNS) in blood culture specimens.

Methods : The Real-MRSA® and -MRCoNS® multiplex real-time PCR assay (M&D, Republic of Korea) uses the following TaqMan® probes which were labeled with different fluorophores (FAM, HEX, and Cy5, respectively): 16S rRNA for *Staphylococcus* species, the *nuc* gene for *S. aureus*, and the *mec*A gene for methicillin resistance. For blood culture, two or three pairs of culture bottles for aerobes or anaerobes were incubated in the BacT/Alert 3D (bioMérieux, Marcy, France), BACTEC™ 9240 system (Becton Dickinson Diagnostic System, Spark, MD, USA), or the BACTEC™ FX (Becton Dickinson) blood culture systems for 5 days after inoculating with blood drawn from the patient at the bedside. The identification of organisms and antimicrobial susceptibility tests (ASTs) were conducted by the microplate method, the MicroScan® system (Siemens Healthcare Diagnostics, Sacramento, CA, USA), and the Vitek® 2 system (bioMérieux, Durham, NC, USA).

Results : The multiplex real-time PCR assay was evaluated using 118 clinical isolates from various specimen types and a total of 350 positive blood cultures from a continuous-monitoring blood-culture system (CMBCS). Cycle threshold (C_t) values were used to determine the limit of detection. The detection limit of the multiplex real-time PCR assay was 10^3 CFU/mL for each gene target. The results from the multiplex real-time PCR assay for the three targets were in agreement with those of conventional identification and AST methods except for one sample. The sensitivities of the multiplex real-time PCR kit were 100% (166/166), 97.2% (35/36), and 99.2% (117/118) for 16S rRNA, *nuc*, and *mec*A genes, respectively, and the specificities for all three targets were 100%.

Conclusion : The Real-MRSA® and -MRCoNS® multiplex real-time PCR assay is very useful for the rapid and accurate diagnosis of staphylococcal blood stream infections (BSIs). Moreover, the multiplex real-time PCR assays may provide the essential information to accelerate therapeutic decisions for earlier and adequate antibiotic therapy based on detection of the *mec*A gene.

B-047

Field Test of the Dynex M² Multiplexed Assay System in the Democratic Republic of Congo Using Dried Blood Spots

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Background: The Dynex Technologies, Inc. M²® multiplex chemiluminescent immunoassay platform was selected as the processing platform for an MMRVT immunity assessment in support of the 2013 Democratic Republic of Congo Demographic Health Survey (DRC-DHS). Within five months and in collaboration with University of California, Los Angeles, Fielding School of Public Health (UCLA-FSPH) Dynex was able to deliver a fully functional automated processing system, reagents and adequate assay plates to process 10,500 dried blood spot (DBS) samples to Kinshasa, DRC.

Methods: Polystyrene beads coated separately with antigen to Measles, Mumps, Rubella, Varicella-Zoster Virus and Tetanus were immobilized within 54-well M² assay strips with 10 beads per well. Three separate within-well positive control beads were coated with horseradish peroxidase, total human IgG, and polyclonal goat anti-human IgG. Two negative control beads were coated with MRC-5 and E6 cell lysate. 423 dried blood spots were anonymously collected from children visiting Kinshasa health centers during the pilot study and more than 8,500 samples collected during the nationwide principal DHS survey. Positive control DBS were made using a 5-donor pool of normal defibrinated serum. Negative control DBS were made from pooled normal IgG-stripped serum. Each DBS was extracted into 1ml of PBS, 0.5% tween20, 5.0% dried milk and processed on a modified Dynex automated DS2® ELISA processing system. Optimization runs in the DRC included examination of DBS spotting order, DBS extraction time, two different anti-human IgG-HRP conjugates, PBS vs. Tris-NaCl wash, room temperate vs. 37°C sample/conjugate incubation temperatures, and 30 vs. 60 minute sample/conjugate incubation times. Duplicate DBS reference sets were made using a 32 previously-characterized plasma samples and a 7-point 4-fold dilution series of pooled positive control into negative serum. The duplicate reference sets were processed independently in Kinshasa and the Dynex labs.

Results: During the initial optimization of the M² testing platform all samples were tested in replicate and gave excellent concordance of clinical calls regardless of processing variables used. Currently, all 423 pilot samples and 1000 DHS samples have been processed. Sensitivity and specificity of the M² system in Kinshasa was shown to be equivalent to that at Dynex based on extraction of the 32-member reference set as well as to fresh dilutions of the control sera. Extraction of the 7-point DBS calibration series in the DRC shows an equivalent assay response to the same set extracted in the Dynex labs. Samples tested in replicate during optimization runs in Kinshasa gave 92% concordance of clinical calls regardless of processing variables used, with discrepant results found within the indeterminate range.

Conclusion: As shown by the speed of assay development, having been deployed to a substantially resource-limited environment, and agreement of replicates regardless of processing conditions the Dynex M² multiplex immunoassay system has shown itself to be a very robust assay platform with excellent sensitivity and specificity. The use of this system in conjunction with DBS processing offers a very cost-effective automated multiplexed immunoassay processing system in challenging environments.

B-048

Laboratory diagnosis of viral respiratory tract infections in a Children's Hospital in São Paulo, Brazil, one year study

L. R. Almeida. *DASA, São Paulo, Brazil*

Background Viruses are recognized as the major cause of respiratory tract infections, particularly in children. Emerging virus, such as Influenza H1N1, Metapnenumovirus and Bocavirus are detected by Molecular Biology methods, with high sensitivity and specificity. Frequently more than one virus are detected in the same sample and considered responsible for these infections.

Methods: Data from the results of laboratory tests for viral respiratory infections were collected, from January to December 2013, for patients attended a children´s hospital in the city of São Paulo Brazil. The test utilized was the RT-PCR Microarray: CLART® Pneumovir virus panel that detects Influenza A, Influenza A H1N1 strain 2009, Influenza B, Parainfluenza1, 2, 3 and 4, Syncycial Respiratory Virus (SRV) A and B, Adenovirus, Bocavirus, Metapneumovirus, Coronavirus, Enterovirus and Rhinovirus.

Results: 1394 respiratory samples were tested by the respiratory virus panel and 67% of the tests were positive for at least one virus. The months with higher positivity were from March to July corresponding to the beginning of autumn and winter, respectively, in Brazil. April showed the major positivity, 87% when compared with the other months. The most frequent viruses identified in this period of time were SRV 36%, Bocavirus 14%, Metapneumovirus 7,2% and Adenovirus 6,3%. The samples were collected from children aged 0 to 14 years and the positivity was higher in young children under 2 years old with 80% of the positive samples. Influenza A H3N2 was detected in two samples during the year 35 samples were positive for H1N1. Most of he results were provided to the physician in two days after collection.

Conclusion: The viral molecular panel detected a wide range of respiratory virus with high sensitivity, including more than one virus in the same sample. The rapid result, two days, is important for the etiologic diagnosis of respiratory infections and infection control measures for the patients admitted to the hospital.

B-049

The First Isolates of the Emerging New Delhi Metallo-β-lactamase in a Laboratory in Rio de Janeiro, Brazil

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Background: Antimicrobial resistance is a growing global challenge to human health. The emerging New Delhi metallo-β-lactamase (NDM), an acquired class B carbapenemase has gain public attention due to its extended hydrolysis of β-lactams including carbapenems. Objective: We report the first isolates of this emerging resistance mechanism in our hospitals in Rio de Janeiro, Brazil. Methods: Between September and October 2013, four carbapenem-resistant strains, three Enterobacter cloacae and one Providencia rettgeri were isolated in our clinical Microbiology Lab from distinct patients hospitalized at different hospitals located at two distinct cities of Rio de Janeiro. MIC was determined by CLSI broth microdilution method. Specific primers were used for PCR detection of blaNDM, blaKPC, blaGES, blaSPM, blaGIM, blaSIM, blaCTX-M, blaSHV, blaTEM, blaOXA-48, armA, rmtA, rmtB, rmtC, rmtD, rmtG, npmA followed by DNA sequencing. Clonal relatedness among E. cloacae isolates was examined by PFGE. Plasmid extraction was performed by Kieser protocol. Conjugation with E. coli J53 (LacZ− Nalr Rifr) and hybridization with specific probes were used to determine transfer of carbapenem resistance. The species identification was confirmed by MALDI-ToF MS® and 16sRNA DNA sequencing. Results: Of the four isolates, three were from public hospital and one from a private hospital. Enterobacter cloacae were isolated in blood, ascitic fluid and rectal swab and one Providencia rettgeri was isolated in urine. All strains showed higher resistant rates to carbapenems and to broad-spectrum cephalosporins and P. rettgeri was resistant to polymyxin B, as expected. blaNDM-1 and blaTEM-1 were identified in all isolates. All E. cloacae also produced blaCTX-M-15, and showed different PFGE pattern. blaKPC-2 was identified in one E. cloacae isolate (isolate E134) and armA gene was detected in E. cloacae gentamicin and amikacin resistant (isolate E133). Conjugation of blaNDM-1 was achieved in 2/4 isolates, E. cloacae (isolate E134) and P. rettgeri (isolate E132). The hybridization revealed that blaKPC-2 was located in the E. cloacae chromosome. The genetic location of blaNDM-1 is being confirmed among the isolates evaluated. Conclusion: Those isolates in Rio de Janeiro, showed the importance of correct molecular study of isolates that express carbapenenem resistance in the clinical Microbiology Lab, as, although the blaNDM-1 has been previously reported in Enterobacteriaceae clinical isolates in our country, this study constitutes the first one that identified the co-association of blaNDM-1 and blaKPC-2 in E. cloacae. The results can lead to improve infection control measures to avoid its spread in hospital environment.

B-050

New materials for Hepatitis A and Hepatitis B IgM immunoassay calibration and quality control

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Purpose: We propose that human monoclonal antibodies can be used as replacements to human serum as controls and calibrators in diagnostic assays for Hepatitis A IgM or Hepatitis B core IgM immunoassays . These *in-vitro* produced, standardized products offer an unlimited and consistent supply of antibody for calibration and quality control of infectious disease immunoassays. Relevance: Despite advances in public health and medicine, infectious diseases are persistently counted as a significant cause of human illness and economic loss. Assays developed for diagnosis and monitoring infectious diseases require robust, stable and readily available control and calibration materials. Traditionally manufacturing of these vital materials has depended upon discovering source plasma units from naturally infected individuals. This material is increasingly difficult to find. We present data on a new source of material for the manufacture of controls and calibrators for Hepatitis A Virus VP1 IgM or Hepatitis B virus core p22 IgM antibodies from immortalized human lymphocytes. Method: Human lymphocytes from individuals expressing the antibody of interest are isolated from fresh whole blood by Ficoll. Following *in vitro* immortalization with Epstein-Barr virus, primary B cells expressing the antibody of interest are fused with a hybridoma partner. Following an extended growth period hybridomas are screened to determine if antibody is being secreted. Monoclonality is assessed by the limiting dilution method. Stability of hybridoma cell lines is assessed and validated through extended cell culture & passage. Results: Recovery parallel to human serum, antibody specificity, lack of cross reactivity and performance in several analytical techniques are shown. Conclusion: These new materials can be used in the formulation of both calibrators and positive controls by manufactures of diagnostic kits for the detection of IgM antibodies to Hepatitis A or Hepatitis B. :

B-051

Vitamin D and Vascular Endothelial Growth Factor Levels in Hepatitis B Infection

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Background: In addition to its well-known effect on calcium metabolism, vitamin D has various roles such as regulation of inflammatory processes, immune response, cell proliferation and differentiation. Hepatitis viruses can cause inflammatory liver disease and vitamin D deficiency was reported in patients with hepatitis C virus (HCV) infection. Vitamin D deficiency is a common finding in chronic liver disease patients but there is not much data on serum vitamin D levels of hepatitis B virus (HBV) infected patients. Angiogenesis may be observed during inflammatory processes and vascular endothelial growth factor (VEGF), which is an important mediator in angiogenesis, is found to increase in mesentery and liver tissue in cirrhotic patients. There are different findings on the effect of vitamin D on VEGF expression in viral hepatitis patients and we aimed to evaluate the relationship between these two markers in HBV infected patients.

Methods: The study included 57 patients with HBV infection and 19 age-matched healthy controls. Serum 25-OH vitamin D levels were measured by Advia Centaur XP chemiluminescence assay (Siemens AG, Germany). Serum VEGF levels were measured by enzyme linked immunosorbent assay (R&D Sytems, MN, USA). Two pathologists evaluated liver tissue samples from HBV infected patients. All data were analyzed using MYSTAT version 12 (SYSTAT, CA, USA). Data is presented as mean ± standard deviation. Spearman's rho and Mann-Whitney U tests were used as appropriate. A test result of $p<0.05$ was considered statistically significant.

Results: Mean serum vitamin D levels were lower in HBV infected patients by 3.79 ng/mL (27%) compared to the controls ($p<0.037$). Serum VEGF levels did not show significant difference between groups. There was no correlation between VEGF and vitamin D levels in patient population, however, control group showed an inverse correlation between these markers ($r2=0.228$, $p<0.039$).

Conclusion: Vitamin deficiencies are common in various viral hepatitis types and we showed that the serum vitamin D levels of HBV patients were lower than controls. Vitamin D is suggested to decrease viral replication so maintaining normal vitamin D levels might be beneficial in viral hepatitis. Both HBV and HCV genes can lead to an increase in the expression of VEFG. Vitamin D and its analogs modulate angiogenesis in viral hepatitis and suggested to be a regulatory factor of VEGF production. The negative correlation, which is found between serum vitamin D and VEGF levels in controls, was not observed in our patient group. This discrepancy might be caused by low vitamin D levels. We advocate measuring vitamin D levels in HBV infected patients; furthermore, we suggest vitamin D supplementation in deficient individuals.

B-054

Use of the MagArray Immunoassay System as a Platform for Pathogenic Escherichia coli Detection

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Background: In recent years, pathogenic Escherichia coli have been causing numerous foodborne outbreaks leading to mild to bloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and even death of patients. Many foods with short shelf life are related to the public before a negative testing for E. coli is confirmed. Currently, the process by which regulatory agencies screen for pathogenic E. coli in foods takes over 3 days. The MagArray immunoassay system is a low-cost chip-based platform capable of simultaneously detecting up to 80 different analytes in as little as 10 min. The reduction in detection time of pathogenic E. coli can contribute to a faster recall of contaminated foods and can therefore limit the number of individuals ingesting the contaminated food and decrease the total cost of lost productivity and treatment. The objective of this study was to demonstrate on MagArray platform the simultaneous detection of two main types of pathogenic E. coli (i.e., O157 and O145) in ground beef with high sensitivity.

Methods: MagArray chips were first spotted with E. coli O145 and O157 antibodies. The chips were then blocked and ground beef samples were spiked with E. coli O145 and O157 for incubation. After incubating with detection antibodies, magnetic particles were then applied to generate signals. Different concentrations of E. coli were spiked to establish the standard curve and determine assay sensitivities.

Results: In this 2-plex immunoassay in ground beef, detection of E. coli at a concentration as low as 2 cfu/µl was demonstrated. More specifically, for 2 cfu/uL of E. coli O145 and E. coli O157, the inter-run CVs were less than 10% for both types. And the results were compared and agree well with samples spiked to pure buffers. This sensitivity of detection was achieved using a 30-min assay. And the results showed that assay sensitivity is minimally affected by changing assay media from pure buffer to ground beef.

Conclusion: The MagArray technology demonstrated that it can provide exceptional sensitivity with reasonable reproducibility for simultaneous detection of both E. coli O145 and O157 in ground beef. Thus we believe this technology provides a good fit for detecting multiple E. coli serogroups. This assay not only accelerates identification of pathogenic E. coli, but also holds the potential to help regulatory agencies to quickly issue a product recall for contaminated foods.

B-055

Real-time PCR TaqMan assay for Rapid Screening of Sepsis using Positive Blood Cultures

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Background : Sepsis is a lethal medical condition that results from a harmful or injurious host response to infection. Rapid detection of pathogens in blood from septic patients is essential for adequate antimicrobial therapy and prognosis of patients. The aim of this study was the acceleration of detection and discrimination of Gram positive (GP)-, Gram negative (GN)-bacteria and *Candida* species in blood culture specimens by molecular methods.

Methods : The Real-Sepsis® real-time PCR kit (M&D, Wonju, Republic of Korea) uses the following TaqMan® probes: the bacterial 16S rRNA gene for pan-GP, pan-GN and fungal 18S rRNA gene *Candida* species, respectively. For blood culture, two or three pairs of culture bottles for aerobes or anaerobes were incubated in the BacT/

Alert 3D (bioMérieux, Marcy, France), BACTEC™ 9240 system (Becton Dickinson Diagnostic System, Spark, MD, USA), or the BACTEC™ FX (Becton Dickinson) blood culture systems for 5 days after inoculating with blood drawn from the patient at the bedside. The identification of bacteria and antimicrobial susceptibility tests (ASTs) were conducted by the microplate method, the MicroScan® system (Siemens Healthcare Diagnostics, Sacramento, CA, USA), and the Vitek® 2 system (bioMérieux, Durham, NC, USA). For identification of *Candida* species, a VITEK-2 (bioMérieux) YST ID CARD was used.

Results : The Real-time PCR TaqMan assay was evaluated using a total of 62 bacterial reference strains representing 39 of GP, 23 of GN species and 25 fungal reference strains. Subsequently, it was evaluated with 115 clinical isolates, 256 positive blood culture specimens and 200 negative blood culture specimens, and results were compared to those of conventional identification method. The overall sensitivity of the real-time PCR TaqMan assay was 99.6% and the specificity was 89.5%.

Conclusion : The Real-Sepsis® real-time PCR assay could not only differentiate bacterial and fungal from viral and other pathogens, but also can classify Gram staining with a much shorter turnaround time than the gold standard culture method. Furthermore, it could have an important impact on choosing the appropriate antibiotic therapy based on simultaneous detection and discrimination GP-, GN-bacteria and *Candida* species.

B-057

In vitro antimicrobial susceptibility of clinical and environmental strains of *Burkholderia pseudomallei* from Brazil

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Background: *Burkholderia pseudomallei*, the causative agent of melioidosis, is intrinsically resistant to a wide range of antimicrobial agents [1]. Ceftazidime is the drug of choice for treating melioidosis, although carbapenems are indicated for severe infections. Following this initial treatment, an eradication phase is recommended, consisting of prolonged oral therapy with trimethoprim-sulfamethoxazole (SXT) combined with doxycycline or amoxicillin-clavulanic acid (AMC) for up to 6 months [2]. The aim of this study was to determine the antimicrobial susceptibility of clinical and environmental *B. pseudomallei* from Brazil.

Methods: Ten clinical strains of *B. pseudomallei* were included in this study, obtained from the DASA central laboratory at Fortaleza, Ceará, and the others environmental strains were obtained from bacterial collection of Federal University of Ceará. Identification of *B. pseudomallei* was confirmed using an automated VITEK® 2 system, bioMérieux, followed by sequencing of the 16S-23S spacer region. For antimicrobial susceptibility assay, five antimicrobial agents were tested by the microdilution technique according to CLSI guidelines [3].

Results: All MICs determined by the broth microdilution from 20 strains of B. pseudomallei in this study were distributed in MIC50 and MIC90 and as the percentage of sensitivity. The percentage of sensitivity for doxycycline, imipenem and sulfametol / trimethoprim were 100% each and amoxicillin / clavulanate and ceftazidime was 80% and 90% respectively (Table 1).

Conclusion: The current results were compatible with those previously reported in the literature [4,5] and corroborate those of Jenney et al. [4]. The susceptibility of the tested strains appears to be independent of the origin of the isolates (environment or clinical cases).

In conclusion, this work provides knowledge on the antimicrobial susceptibility of *B. pseudomallei* from Brazil, serving as a guide for the selection of appropriate empirical therapy, thus contributing to better medical care in addressing melioidosis.

Antimicrobials	MIC (µg/mL) ^{50}	MIC (µg/mL) ^{90}	Range	Susceptibility (%)
Amoxicilin/ clavulanate	8/4	18/8	4/2 - 32/16	80
Ceftazidime	4	16	2 - 16	90
Doxycycline	0	1	0,25 - 0,5	100
Imipenem	1	1	0,125 - 1	100
Trimethoprim-sulfamethoxazole	1	2	0,125/2,375 – 2/38	100

B-058

The performance of a highly sensitive chemiluminescent enzyme immunoassay for HBsAg.

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[Background] HBsAg is an envelope protein of HBV and is continuously secreted into blood as not only a portion of HBV but also a secretion protein during HBV persistent infection. HBsAg level in blood is correlated with intra-hepatic covalently closed circular DNA (ccc DNA) and is useful as an indicator of HBV persistent infection, especially during treatment with anti-viral drugs.

We have developed a fully automated highly sensitive chemiluminescent enzyme immunoassay for HBsAg (new CLEIA system) which has 10 fold higher sensitivity than a commercially available HBsAg kit. We evaluated the basic performance for the new CLEIA system and here we report the results.

[Methods] The highly sensitive chemiluminescent enzyme immunoassay was run on the fully-automated CLEIA system LUMIPLUSE G1200 (FUJIREBIO INC.).

[Results] The CV for within-run reproducibility was 0.5-3.3% and for between-run reproducibility it was 0.5-1.4%. The quantitation limit was 5 mIU/mL (0.005 IU/mL). The new CLEIA system could detect 1-3 bleeds earlier than the commercial HBsAg kit in seven seroconversion panels among nine. The correlation coefficient and the slope with the commercial HBsAg kit were 0.92 and 1.30, respectively.

[Conclusion] The new CLEIA system has good reproducibility and high sensitivity. And it shows good correlation with a commercial HBsAg kit. In addition, the new CLEIA system can be easily operated to complete an assay in 30 min.

The new CLEIA system is considered quite useful for the routine quantification of HBsAg.

B-059

Utilization of Serum Total Bile Acids for the Prediction of HCV Active Infection in Anti-HCV Antibody Positive Patients

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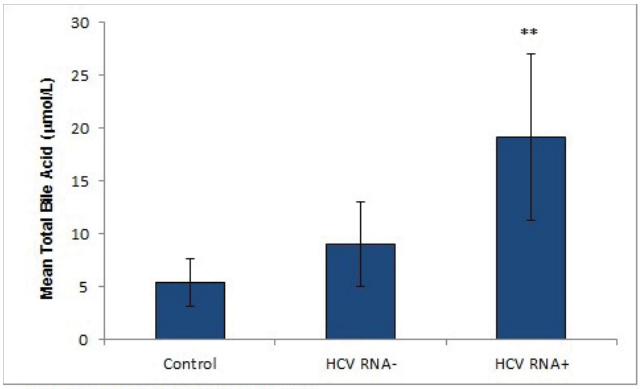
Background: Conventional liver function tests do not correlate with active hepatitis C infection or response to treatment. Recent studies have suggested that bile acids (BA) in the blood may be elevated in patients with detectable HCV RNA levels. It has also been shown that bile acids increase HCV RNA replication and has been suggested as a possible etiology for poor response to IFN therapy in patients with specific genotypes.

Methods: Total BA levels from blood samples were measured on the Beckman DxC using the Diazyme Total Bile Acids Assay. Conventional liver function tests using Beckman reagents were performed on the Beckman DxC. BA levels of 30 anti-HCV antibody positive patients with detectable HCV RNA and 30 without detectable HCV RNA were compared to 24 healthy controls. Reference range includes results < 10 umol/L. Mean values, 95% confidence intervals, and p-values from independent sample Student's unpaired t-test were calculated using MS Excel.

Results: Mean total BA values for controls and HCV RNA negative patients fell within the reference range while mean values for HCV RNA positive patients were elevated. Mean total BA levels were statistically significantly higher in patients with detectable HCV RNA levels versus controls and patients with undetectable HCV RNA levels (p-values: control, 0.09; HCV RNA-, 0.05). No statistically significant difference was observed for liver function test values between the 3 compared groups.

Conclusions: Currently, quantitative HCV viral load testing is employed to monitor treatment response but remains costly and is not practical for surveillance in chronic hepatitis C patients. Our preliminary findings suggest that total BA levels may distinguish between active and chronic hepatitis C infection. For physicians needing a non-invasive and less cost-prohibitive method for monitoring of recurrence or active infection in their hepatitis C patients, bile acid testing may prove a viable tool in their arsenal



B-061

Prevalence of Tuberculosis in Sao Paulo diagnosed by Laboratory tests in the period 2011-2013

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Background: According to data presented by the World Health Organization (WHO) in 2010 were diagnosed and reported 6.2 million cases of tuberculosis (TB) worldwide, with 5.4 million new cases, representing 65% of the estimated cases for the same year. Countries like China and India account for 40% of cases, and Brazil is among the 22 countries which account for 82% of TB cases worldwide.

Combating TB 2011 - 2015 follows the overall plan proposed by the WHO. Your goal is to dramatically reduce the burden of disease by 2015. The main objective to reduce TB are:1) reduce the incidence of TB in HIV / AIDS and the incidence of HIV in TB patients, prevent and control - multidrug-resistant TB and strengthen actions to meet the needs of poor and vulnerable populations, 2) strengthen the health system based on primary care, 3) engage all providers of health services, and 4) enable and promote research and others.

The Plan also has, as main targets: to reduce the incidence and mortality of TB until 2015 compared to 1990 and eliminate TB as a public health problem until 2050. With this goal it becomes increasingly important to accurate and early diagnosis of this disease and laboratory testing and higher efficiency are of great importance in this context.

Objective: To evaluate the percentage of positive diagnosis of TB for each specific laboratory test for this disease for the period 2011 to 2013, retrospectively analyzing the database of a Laboratory Oversize working in São Paulo, Brazil

Material and Methods: The authors retrospectively analyzed 44931 results of laboratory tests ordered for diagnosis and monitoring of TB originated from 42 ambulatory care of Associação Fundo de Incentivo a Pesquisa- Afip, from Sao Paulo- Brazil, (January2011-December2013). Laboratory tests analyzed were: Adenosine deaminase (ADA), Bacillus Koch (BK), BK-automated culture/Bactec , *Mycobacterium tuberculosis* PCR.

Results: The test results of ADA, BK. And BK- automated culture/Bactec from of years (2011, 2012 and 2013) with their respective percentages of positivity were: ADA for 2183 (23.5%), 2105 (21.9%), 2233 (48.8%), search for BK , 7219 (9.5%), 7563 (10.1%), 7400 (10.4%); BK-automated culture/Bactec 1926 (6.0%) 1653 (5.5%) 1535 (8.0%), and *Mycobacterium tuberculosis* PCR, 48 (4.2%) 79 (6.3%) 95 (7.4%).

The data were presented as percentage of positive prevalence of the most requested in the affiliated units of the Afip laboratory. However, these methods have limitations and many of them are carried out in more than one sample.

Conclusion: We note that the ordering patterns of these tests remained constant over the years. The PCR method showed a small increase in use and an increase of positivity but the general results showed that the most requested examination for diagnosis of TB is a direct search in lamina and culture of BK. Analyzing the percentages of positivity of the Afip tests performed, we conclude that from 2011 to 2013 there was an increase in cases of TB of nearly 0.5 to 1.5%, which is very worrying that we may achieve the government's goal to reduce TB cases until 2015.

B-062

Comparison of clinical performances among Roche Cobas HPV, RFMP HPV Papillo Typer and Hybrid Capture 2 assays for detection of high-risk types of human papillomavirus

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Background: High-risk types of human papillomavirus (HR-HPV) is an important cause of cervical cancers. Current cervical cancer screening guidelines suggest that early detection of HPV-16 and HPV-18 may prevent the progression of cervical cancer. We evaluated and compared three HPV DNA tests, Roche Cobas HPV (Roche Molecular Systems Inc., Pleasanton, CA), RFMP HPV Papillo Typer (GeneMatrix Inc., Yongin, Korea) and Hybrid Capture 2 (HC2; Qiagen, Gaithersburg, MD, USA). The HC2 has been recommended for use as a reference test, Roche Cobas HPV specifically identifies HPV-16 and HPV-18 with concurrently detecting other 12 HR-HPV types and RFMP identifies 74 HPV genotypes.

Methods: A total of 861 cervical swab specimens from women over 30 years of age were classified into groups of high grade squamous intraepithelial lesion (HSIL) and non-HSIL according to cervical cytology results and analyzed by Roche Cobas HPV, RFMP HPV Papillo Typer and HC2. The results of direct sequencing or Linear array (LA; Roche Molecular Systems Inc., Pleasanton, CA) HPV genotyping test were considered true when three assays presented discrepancies.

Results: Concordance rates between Roche Cobas HPV *vs.* RFMP, RFMP *vs.* HC2, and HC2 *vs.* Roche Cobas HPV were 94.5% (814/861), 94.2% (811/861), and 95.8% (825/861), respectively. In 71 specimens with discrepant results, concordance rates between each assay and direct sequencing or LA were as follows: Roche Cobas HPV, 35.2%; RFMP, 93.0%; HC2, 25.4%. Clinical sensitivities and specificities for detecting HSIL were 80.3% and 95.8% with Roche Cobas HPV, 83.6% and 95.1% with RFMP and 90.2% and 94.8% with HC2.

Conclusion: Roche Cobas HPV, RFMP and HC2 showed high agreement rates each other. Although Roche Cobas HPV and RFMP showed lower clinical sensitivity in detecting HSIL compared to HC2, they would be clinically useful since both provide HPV genotypes.

B-063

Prevalence of fungal bloodstream infections in a tertiary University Hospital in Brazil - Comparative analysis between two periods in the last decade

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Background: In the last decades, the growing population of immunosuppressed hosts has dramatically increased. Therefore, the prevalence of nosocomial fungemia has increased throughout the world and mortality from this disease is high. The objective of the study is to identify the etiology of fungal bloodstream infections in a Tertiary University Hospital in Belo Horizonte, Brazil, comparing two periods in the last decade.

Methods: we retrospectively analyzed the results of all blood cultures processed in the hospital, between two periods: from 2001 to 2003 and from 2011 to 2013. For each triennium were reported the number of blood cultures collected, the number of positive cases, the percentage of fungemia and all identified fungal species. The samples were observed in the laboratory routine carried out by incubation in BacT ALERT® (bioMérieux). The positive samples were subcultivated for species identification through morphologic and biochemical assays.

Results: From 2001 to 2003, 34.822 blood culture were performed and 5,510 (15.8%) positive. Fungi were isolated in 229 (16.4%) cases. From 2011 to 2013, the number of blood cultures increased to 55,052, but the number of positive samples decreased to 4,873 (8.9%). Fungal bloodstream infections increased to 290 (6.00%) cases. Candidemias were predominant: 97.38% (2001-2003) and 91.72% (2011-2013). The isolated species are shown in Table 1.

Conclusions: The prevalence of fungemia increased in the last decade. Candidemia was responsible for more than 90% of the cases. Non-albicans Candida species increased and C. albicans decreased. Others species of fungi increased too.

Prevalence of fungal species on bloodstream infections in University Hospital in the last decade

Species	2001-2003		2011-2013		Total	
	N	%	N	%	N	%
Candida albicans	91	39.74	84	28.97	175	33.72
Candida glabrata	1	0.44	5	1.72	6	1.16
Candida guilliermondii	3	1.31	3	1.03	6	1.16
Candida kefyr	0	0	2	0.69	2	0.39
Candida krusei	0	0	9	3.10	9	1.73
Candida parapsilosis	60	26.2	76	26.21	136	26.20
Candida spp	29	12.66	24	8.28	53	10.21
Candida tropicalis	39	17.03	63	21.72	102	19.65
Cryptococcus neoformans	3	1.31	7	2.41	10	1.93
Cryptococcus spp	3	1.31	3	1.03	6	1.16
Fusarium sp	0	0	8	2.76	8	1.54
Trichosporon spp	0	0	6	2.07	6	1.16
Total	229	100	290	100	519	100

B-064

Distribution of HIV genotypes among Brazilian regions

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Background: Lung cancer is the most prevalent life-threatening cancer worldwide with more than 80% being non-small cell lung cancer (NSCLC). Detection of mutations of EGFR gene is critical for predicting the response to therapy with tyrosine kinase inhibitors (TKIs), such as gefitinib and erlotinib, in patients with NSCLC. Patients that are EGFR mutants have constitutive TK activity and, therefore, a greater sensitivity to anti-EGFR inhibition.

Objective: To describe the EGFR mutations frequency found in lung adenocarcinoma samples, using pyrosequencing method.

Method: Thirty samples of lung adenocarcinoma were analyzed from January 2013 to December 2013. The test was performed on formalin-fixed, paraffin-embedded tumor specimen, after the selection of the specimen region to be analyzed by a pathologist. The DNA was extracted using the Qiaamp FFPE Tissue kit (Qiagen, Hiden, Germany). Concentration of DNA sample was measured spectrophotometrically using a NanoDrop spectrophotometer (NanoDropTechnologies, Wilmington). Codons 719, 768, 790, 858, 861 and exon 19 were amplified by PCR using the EGFR Pyro kit (Qiagen, Hiden, Germany). Successful and specific amplification of the region of interest was verified by visualizing the PCR product on capillary electrophoresis using Qiaxel DNA Screening Kit (Qiagen, Hiden, Germany). Preparation of single-stranded DNA was done using PyroMark Q24 vacuum workstation (Qiagen, Hiden, Germany) according to the manufacturer instructions. The pyrosequencing reaction was analyzed on the Pyro Mark Q24 (Qiagen, Hiden, Germany)

Results: The frequency of EGFR mutations found is presented on Table 1. All mutations together represent only 27% of the samples.

Conclusion: The results are consistent with previous studies and reports. The single-point mutation L858R (CTG> CGG) on exon 21 and the frame deletions on exon 19 represents the majority mutations found in Brazilian lung adenocarcinoma samples, although most samples showed no mutation at the target regions.

Table 1. Frequency of EGFR mutations found in lung adenocarcinoma samples.

Results	**Frequency**
Wild type	73%
2235del15 (exon 19)	3,3%
2236del15 (exon 19)	3,3%
2237_2255>T (exon 19)	3,3%
2239_2248>C (exon 19)	3,3%
CTG>CAG (L861Q)	3,3%
CTG>CGG (L858R)	10%

B-065

Soluble CD14-subtype, a possible new biomarker increases in septic patients' plasma from pediatric department.

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Background: Soluble CD14-subtype, named presepsin (P-SEP) is a fragment of CD14 peptide produced through phagocytosis of microorganisms by neutrophils. Increased serum concentration of P-SEP was reported in adult patients with severe bacterial sepsis (Shozushima T, et al. J Infect Chemother 2011;17:764-9), however, there have been limited reports on pediatric patients. In order to clarify the significance of P-SEP as a marker of septic disease in children, we conducted a study of serum P-SEP concentration in pediatric patients with febrile diseases.

Methods: Forty-eight children (29 males, 19 females, 0.6 to 152 months after birth, mean age 2.43 years old) admitted to our hospital were enrolled. Plasma was obtained within 24 hours after blood withdrawal. P-SEP was assayed using PATHFASTTM chemiluminescent enzyme linked immunoassay system (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). This automatic analyzer enables to get results within 20minutes. Procalcitonin, white blood cells and C-reactive protein concentration were assayed simultaneously. The ethic committee of Showa University Northern Yokohama Hospital approved this study.

Results: P-SEP concentration was 442 plus minus 301 ng/L (mean and SD) in patients whose blood culture was positive on admission (n=4). For example, staphylococci were detected with blood culture from a 30 months-old female patient. Her P-SEP concentration was 866 ng/L on admission, then decreased after antimicrobial treatment to 235 ng/L when she was discharged. P-SEP concentrations were 191 plus minus 47 in viral infections (n=9), 313 plus minus 90 ng/L in Kawasaki's disease (n=6). On the other hand, cases with blood culture negative but urine and/or sputum culture positive showed 349 plus minus 202 ng/L (n=9). Other culture negative patients (n=20) showed 267 plus minus 132 ng/L.

Discussion: P-SEP has been reported to be an indicator of prognosis in adult septic patients (Masson S, et al. Crit Care 2014;18:R6), and critically ill preterm newborns (Mussap M et al. J Matern Fetal Neonatal Med 2012;25:51-3). Though statistically not significant, plasma P-SEP was higher in septic children compared to those without bacterial infections. Reference interval of plasma P-SEP concentration in adults under the age of 70 is ranged 201 to 457 ng/L (Chenevier-Gobeaux C, et al. Clin Chim Acta 2014;427:34-6). Our study suggests reference interval in children is likely to be lower than that in adults. More study is required to confirm the results.

Conclusion: Increased plasma concentration of P-SEP was observed in pediatric patients with bacterial sepsis. P-SEP could be a possible biomarker of sepsis in pediatric patients.

B-066

Analysis of blaKPC gene from Hodge Test screening confirming KPC enzyme resistance

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Background:The Klebsiella Pneuminiaie Carbapenemase (KPC) is responsible for human infections, especially in hospital environment. It is an enzyme produced by Gram-Negative bacilli and its detection in bacterial isolates confers resistance to the carbapenem antibiotics and furthermore inactivates penicillins, cephalosporin and monobactams.The transmission, in hospital environment, occurs through the contact between secretion from infected patients. The objective of this study was to evaluate the correlation of positive results on the Hodge Test with detection of the blaKPC gene confirmed by Molecular Biology (Life Technologies - Real Time PCR System 7300).

Methods: 105 cultures from samples of LANAC Laboratory of different materials were selected and the resistance profile was observed to multiple antibiotics (especially carbapenems) using the method of disk diffusion and automated system MicroScan WalkAway - Siemens Healthcare Diagnostic during 2011 and 2012.

Results:Multidrug resistant strains are not new or specific for Klebsiella species. In 100 of 105 analyzed cultures *K. pneumoniae* was isolated (95.2%). 98% of these isolates showed concordance between Hodge Test and the results obtained by Life Technologies - Real Time PCR System 7300 (blaKPC gene detectable). 2% of the isolates were indeterminate by Hodge Test. The others microorganisms isolates on the analyzed cultures were *E. cloaceae* (2,8%); *Enterobacter sp* (0.95%) and *Proteus vulgaris* (0,95%). In only two samples the correlation between the tests were not confirmed.

Conclusion: In this study was observed 98% of correlation between Hodge Test and the detection of blaKPC gene by Molecular Biology (Life Technologies - Real Time PCR System 7300). The determination of the Minimum Inhibitory Concentration (MIC) using the equipment MicroScan WalkAway - Siemens obtained an excellent performance in the correlation between positive Hodge Test and detectable blaKPC gene by Molecular Biology (Life Technologies - Real Time PCR System 7300). Hodge Test and microbiology system automation seems to be an excellent alternative for clinical laboratory routine with high sensitivity and lower cost.

B-067

Ziehl-Neelsen staining as an aid in screening for diagnostic of systemic fungal infection.

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Background: Mycology is still an area that has little clinical importance, although the number of susceptible patients to fungal infection arises through the years. Every single day, in laboratory cytology routines, sputum samples are collected for Acid-fast stain (Ziehl-Neelsen method) for the differential staining procedure to members of the genera mycobacteria (M. tuberculosis, M. leprae), bacteria (Nocardia) and fungus (Cryptosporidium,). It's known that many systemic fungal infections are similar to other common pulmonary diseases, and the differential diagnostic is difficult. We have tried, through a visual screening of compatible fungal structure, to identify medically significant fungi, to an additional specific Mycosel agar culturing step. Although Ziehl-Neelsen is not intended to staining of fungal genera, we thought if it could also be used for the primary identification of fungal pathogens.

Methods: The test was performed with routine sputum samples from Laboratório Alvaro (DASA group), collected by spontaneous or induced expectoration and kept under refrigeration of 2 - 8°C. These samples were primary intended to Ziehl-Neelsen staining procedure for identification of M. Tuberculosis. After staining and visual observation of fungus structure, the cytologist is capable of reporting if a fungal infection is or is not present in the sample. After visual inspection, 150 potential positive samples were selected. Mycosel Merck culture medium was prepared by dilution as described in technical data sheet. This medium is specific for isolation of pathogenic fungi. After inoculation, samples remained in an incubator set to 35 ° C for approximately 30 days. After the incubation time, each grown fungal structure was identified by slide morphological observation, in which Cotton blue staining (specific for examination of fungal colonies) was applied.

Results: From 150 samples, we had no growth in 14% (21/150), 86% (129/150) were positive, where 55% (37/150) corresponding to C. albicans yeast, 9% (6/150) to C tropicalis, 5% (3/150) C. glabrata, 5% (3/150) of yeast and hyphae C. albicans, 1% (1/150) C. parapsilosis, 1%(1/150) Nigrospora and 1% (1/150) of Candida Krusei. Although there is significant positivity for Candida genera, it can't be easily implicated in systemic fungal infection, as opposed to 9 % (6/150) of fungal, normally associated to pulmonary disease. Our final finding was 4% of Histoplasma sp, 3% Aspergillus sp, 2% of Paracoccidioides sp, fungus that are morphologically classified as positive for severe pulmonary disease.

Conclusion: This study provides evidence of the presence of etiologic agents of severe pulmonary fungal disease in sputum of patients originally submitted to Acid-Fast staining. The simple screening to fungal structure in Ziehl-Neelsen stained slides, have shown to be applicable, simple and effective to directing potential positive samples to further culturing in selective medium for isolation of pathogenic fungi. This new procedure can be meaningful in evaluating TB like suspect patients not just on the basis of symptoms, clinical signs, but providing another reliable screening tool.

B-068

Development of a Point-of-Care Diagnostic for Ebola and Sudan Virus Detection

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Background: Viral hemorrhagic fevers are serious, often fatal illnesses characterized by high fever, damage to the vascular system, and multi-organ failure. Because of their rapid progression, the ability to detect and distinguish hemorrhagic fevers is paramount to treatment and survival. To this end, we report on the characterization of rapid point-of-care diagnostic tests for Ebola (EBOV) and Sudan (SUDV) virus detection.

Methods: Using recombinantly produced proteins, we generated a library of polyclonal and monoclonal antibodies recognizing EBOV and SUDV GP, NP, and VP40. Antibodies were initially tested using a multivariate approach with each antibody being tested for use as both capture and detection capability. Testing was performed over a range of plate coating concentrations, nitrocellulose dot blots and stripings, HRP and gold conjugation conditions, and sample dilution ratios. The pairings were further optimized by testing the EBOV and SUDV plate coating concentrations and HRP-conjugate dilutions against various sample dilution titrations to determine the conditions that favored sensitivity and signal. Testing was performed using chosen antibody pairings for the EBOV and SUDV ELISA to confirm that the pairings are optimal by running dose-response curves of both purified EBOV VP40 or SUDV VP40 antigen spiked into a normal human serum control matrix, determining the signal to noise ratio, linear range, LOD, LOQ, and LOB. Candidate antibody pairings identified during the antibody screening process for use on the lateral flow immunoassay (LFI) format were conjugated to gold nanoparticles and striped onto nitrocellulose using the Biodot XYZ dispenser. Testing was performed using purified antigen spiked into normal human serum control matrix.

Results: As demonstrated by ELISA, we found polyclonal antibody pairings against EBOV and SUDV VP40 to be the most reliable in detecting purified recombinant protein in spiked samples. Pairings exhibited limits of detection as low as 10ng/mL, and limits of quantitation ranging from 10-100ng/mL, suggesting that these critical reagents possess the ability to detect low amounts of EBOV and SUDV protein. Pairings migrated to the LFI test strips showed the ability to detect both EBOV and SUDV VP40 recombinant protein in a dose-dependent manner down to 100ng/mL within 10 minutes. Importantly, this dose-dependency was distinguishable with the naked eye, indicating the utility of this rapid test in environments lacking conditioned power and/or significant medical training.

Conclusions: We have developed and characterized prototype ELISA and LFI tests capable of detecting EBOV and SUDV proteins in sample matrix. In the ELISA format, multiple pairings were able to detect EBOV and SUDV VP40 antigens in spiked matrix with acceptable sensitivity, suggesting that with further optimization and ELISA test for the detection of EBOV and SUDV is within reach. In the LFI platform, two pairings showed the ability to detect EBOV and SUDV antigens in a concentration-dependent manner. Importantly, this concentration dependency was discernible without the aid of any instrumentation, suggesting a path forward for the optimization of a rapid, point-of-care test that can be used in austere environments.

B-069

Heparin Binding Protein for Discrimination of Infected and Non-infected Critical Ill Patients from Cardiovascular Conditions - Results of a Pilot Evaluation

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Background Heparin binding protein (HBP) is an inflammatory mediator released into the circulation during neutrophil activation. HBP has been shown to contribute diagnostic information to the differentiation of viral and bacterial infections. Bacterial infection is the most important trigger for the development of sepsis. Especially in critical ill patients the early detection of infection is necessary for appropriate treatment. We thought to investigate whether HBP is able to detect bacterial infection in critical patients from cardiovascular conditions admitted at the intensive care unit (ICU).

Methods 20 patients admitted at the ICU with severe cardiovascular conditions were included. 12 patients developed additional infectious diseases of whom 4 patients developed sepsis. Serum HBP concentrations were measured using the Heparin Binding Protein EIA (Axis-Shield Diagnostics Ltd. Dundee). C-reactive protein (CRP) was determined using the cobas assay (Roche Diagnostics).

Results The discrimination of HBP and CRP concentrations between patients with (n=12) and without infection (n=8) was examined by Mann-Whitney independent sample test. The results are displayed in the table.

Tab. 1: HBP and CRP values in ICU patients with cardiovascular conditions with and without additional infectious diseases

	Without infection, n=8 Median (IQR)	With infection, n=12 Median (IQ)	p value
HBP, µg/L	60 (39-95)	145 (121-238)	0.0087
CRP, mg/L	72 (34-124)	159 (99-206	0.0136

The determination of HBP in serum provided a higher significance level for differentiation between patients with and without infectious diseases compared to CRP. These results could be confirmed by ROC analysis yielding area under the curve (AUC) values of 0.854 and 0.833 for HBP and CRP, respectively. Logistic regression analysis with HBP and CRP as independent variables revealed an AUC value of 0.906 demonstrating that the simultaneous determination of HBP and CRP provided additional diagnostic information.
Conclusion HBP allows highly significant discrimination between infected and non-

infected critical ill patients with cardiovascular complications admitted at the ICU which was superior compared to CRP. Additionally, simultaneous determination of HBP and CRP showed higher diagnostic efficacy than both markers alone.

B-070

Diagnostic Evaluation of Focus Diagnostics Simplexa™ Dengue real-time polymerase chain reaction (RT-PCR) detection and typing of dengue virus

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Dengue is the most important arthropod-borne viral infection of humans and the incidence of dengue has grown dramatically. Dengue virus (DENV) infection affects over 40% of the world's population. Worldwide, an estimated 2.5 billion peopleare at risk of infection.

Dengue viruses belong to the genus flavivirus within the *Flaviviridae* family. The virus group consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that manifest with similar symptoms. DENVs produce several syndromes that are conditioned by age and immunological status.Laboratory confirmation of dengue infection is crucial as the broad spectrum of clinical presentations can make accurate diagnosis difficult.

Dengue can be diagnosed by isolation of the virus, by serological tests, or by molecular methods.Seroconversion of IgM or IgG antibodies is the standard for serologically confirming a dengue infection. Viral antigens also provides evidence of infection and virus isolation provides the most specific test result.The RT-PCR and other PCR-based techniques have become a primary tool to detect virus in the early course of illness. In addition, molecular testing allows the monitoring of outbreaks by detecting the emergence of new serotypes, thus permitting the implementation of control measures.

Therefore, the aim of the project was to evaluatethe diagnostic accuracy of the commercially available Focus Diagnostics Simplexa™ Dengue real-time polymerase chain reaction (RT-PCR) assay for the in vitro detection and typing of dengue virus serotypes 1, 2, 3 and 4 and compare with results obtained from serology.

The RNA of 37 IgM and/or IgG positive samples were extracted using the QIAamp RNA Viral Kit (Qiagen, Germany) according to the manufacturer's recommendations. The amplification of the extracted RNA used bi-functional fluorescent probe-primers and reverse primers. The assay amplifies four serotype specific regions: dengue 1 (NS5 gene), dengue 2 (NS3 gene), dengue 3 (NS5 gene) and dengue 4 (capsid gene). An RNA internal control was used to monitor the extraction process and to detect RT-PCR inhibition. A positive control for all four serotypes was added during the extraction and RT-PCR reaction.

We tested 37 serologically positive samples. In order to compare serologywith RT-PCR, any samples positive for IgM and/or IgG were considered a 'positive' diagnosis of dengue. Of the 37 samples tested from patients with positive serology, 8 (21.62%) were found positive by RT-PCR, with Ct values ranged between 30.3 to 39.6. All positive RT-PCR samples were IgM positive and were negative for IgG. The assay did not detected viral RNA in positive IgG sample (32.43%).

Serological assays are commonly used for diagnosis of dengue infection, as they are relatively inexpensive and easy to perform. However, the detection of antibodies in a dengue-infected person is only possible after 4-5 days of disease onset. One advantage of the RT-PCR assay is the ability to detect and serotype viral RNA early in dengue illness, which is important to diagnosing acute infection and provides the opportunity to impact patient management.

B-071

Analytical Reactivity and Preliminary Performance Results of the BD MAX™ QS Vaginal Panel*

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***The BD MAX™ QS Vaginal Panel is not available for sale or use in the U.S**

Background: The BD MAX™ QS Vaginal Panel is an automated qualitative *in vitro* diagnostic test for the direct detection of *Candida* species associated with vulvovaginal candidiasis (VVC), trichomoniasis, and bacterial vaginosis (BV) from vaginal swabs in women with clinical symptoms of vaginitis/vaginosis. The test utilizes real time PCR for the detection and identification of organisms. This study aimed to (1) challenge the assay with a wide range of strains in an analytical reactivity (inclusivity) study, (2) evaluate the capacity of the assay to detect targets during a co-infection and potentially support the rationale for patient treatment decisions and (3) collect preliminary results from clinical specimens tested with the BD MAX™ QS Vaginal Panel on the BD MAX™ System.

Methods: An analytical reactivity study was performed in the presence of simulated vaginal matrix, on a minimum of 5 strains for each cultivable organism (58 strains total), originating from 12 countries. The capacity to detect co-infection was tested using two combinations i.e. low load of *Trichomonas vaginalis* (TV), *Candida glabrata* and *Candida krusei* in the presence of a high load of *Candida albicans* and low load of TV in presence of high load of *C. glabrata*. Vaginal swabs collected from women with vaginal symptoms were characterized using various reference methods and were then tested with the BD MAX™ QS Vaginal Panel. In Pouch™ TV test was used as the reference method for TV while culture followed by BD Phoenix™ identification was used for *Candida* species and the Nugent Score was used as the reference method for BV. Amsel's Criteria were used to provide a final result for specimens with intermediate Nugent Score. The preliminary performance of the assay for detection of trichomoniasis, *Candida* species associated with VVC and BV was established using a Receiver Operating Characteristic (ROC) curve analysis. The diagnosis of BV was determined using an algorithm based on PCR parameters for the detection of five BV associated markers (*Lactobacillus* species, *Gardnerella vaginalis*, *Atopobium vaginae*, BVAB-2 and *Megasphaera-1*).

Results: The assay identified all strains tested for each analyte in the inclusivity study. Simulated co-infection studies demonstrated the capacity of the assay to detect low loads of a specific organism in the presence of high load of another organism. The preliminary assay performance results (sensitivity/specificity) based on analysis of 771 characterized clinical samples were as follows: TV (94.4%/100%), Candida species (86.8%/94.8%), Bacterial Vaginosis (91.9%/86.2%).

Conclusion: The BD MAX™ QS Vaginal Panel demonstrated high levels of detection for BV, trichomoniasis and *Candida* species associated with VVC simultaneously from vaginal specimens.

B-072

A fast and sensitive (1→3)-β-D-glucan microfluidic assay for the diagnosis and treatment monitoring of invasive fungal infections

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Incidence of invasive fungal infections (IFIs) is on the rise in recent decades with increasing morbidity and mortality rate in many critically ill patients. Diagnosis is often difficult with conventional methods, including biopsies (risk of complications), imaging (nonspecific and limited use for early detection), and culture (slow, high rate of false negative). Without proper diagnosis and treatment, IFI patients may die within weeks.

(1→3)-β-D-glucan (BDG) is a major cell wall component of pathogenic fungi (e.g. Candida, Aspergillus, Fusarium, Acremonium and Pneumocystis), and the increase of BDG concentration in blood has been correlated to fungal infection in patients. Currently, BDG assays are based on the recognition of BDG by coagulation factor G from horseshoe crab amebocyte lysate. We have used a recombinant β-glucan recognition protein (rBGRP) that contains the binding domain of factor G to develop a liquid-phase binding electrokinetic analyte transport assay (LBA-EATA) for BDG in serum.

Our LBA-EATA assay takes advantage of some inherent features of a micro total analysis system (μTAS), including shorter reaction time, low reagent consumption, and minimal reagent and sample handling. In a microfluidic chip channel, the complex of BDG bound by DNA-rBGRP to speed complex migration and fluorescent dye conjugated rBGRP for detection is concentrated by isotachophoresis (ITP) to enhance detection sensitivity. The concentrated complex is subsequently separated from noise signals in another part of the chip channel by capillary zone electrophoresis (CZE) and detected by laser induced fluorescence (LIF). The ITP-CE process is completed within 3 minutes, and the assay can detect BDG in clinical specimens in the low picogram per milliliter range (~ 10pg/ml). This sensitivity is sufficient to differentiate BDG in healthy human population (10-40 pg/mL) and should be capable of detecting the early onset of fungal infections when used in conjunction with other diagnostic methods.

B-073

Multicenter Evaluation of Mindray Fourth-generation CL-2000i HIV Ag/Ab Combination Assay

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Background: The Centers for Disease Control and Prevention (CDC) recently proposed to use fourth-generation HIV immunoassays for screening. Mindray CL-2000i HIV Ag/Ab Combination assay (CL-2000i) is a newly developed fourth-generation HIV assay that simultaneously detects HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2. The objective of this study is to evaluate the performance of CL-2000i via a multi-center study in three clinical trial centers and National Institutes for Food and Drug Control (NIFD) of China on well-characterized specimens.

Methods: The evaluation was performed on 635 HIV-infected and 1793 HIV-uninfected specimens at three clinical trial centers and NIFD. HIV-infected specimens were either confirmed with nucleic acid amplification testing (NAT), or repeatedly reactive by other chemiluminescence immunoassays and clinical diagnostics. Positive samples of antibodies to HIV-2, HIV-1/O, p24 antigens, and seroconversion panels are obtained commercially. All samples were tested by CL-2000i in comparison with ARCHITECT.

Results: The sensitivity of CL-2000i was 100% for antibodies of HIV-1 (635/635; 95% confidence interval: 99.40 - 100.00%). All the positive samples of the following analytes were reactive: antibodies to HIV-1 Group O (5/5) and HIV-2 (30/30), and HIV p24 antigen (23/23). The specificity of the assay was 99.83% (1790/1793; 95% confidence interval: 99.51 - 99.94%). Testing of 13 HIV-1 seroconversion panels indicates a comparable power of detecting acute HIV infection between CL-2000i and ACHITECT. In each of 3 seroconversion panels, CL-2000i can detect one more positive sample than ACHITECT, equivalent to at least 2 days earlier detection. This was attributed to the high sensitivity of HIV-1 p24 antigen (< 0.25 IU/mL, the most sensitive p24 assay in the market). One HIV antibodies negative sample determined by third-generation HIV EIA assays are strongly reactive with both CL-2000i and ACHITECT, indicating the power of detecting HIV p24 antigen by the fourth-generation HIV Ag/Ab combination assays.

Conclusion: CL-2000i exhibits high sensitivity and specificity, and the ability of early HIV detection. It can detect all the available known antibody positive samples for HIV-1/M, HIV-1/O, HIV-2, and HIV p24 antigen. It is well suited for screening of early HIV infection.

B-074

Use of an Integrated Molecular Diagnostic Platform with a Diverse Array of Specimen Types To Address Laboratory Automation Needs

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Introduction: Molecular methods have revolutionized the way clinical labs identify the presence of microorganisms in patient samples, monitor viral responses to therapy, and characterize genetic disorders. With the demand on test menu expansion, and common limitations in lab space, sample flexibility, budget for new instruments and time for operator training, molecular laboratories require greater platform consolidation. In addition to this, automated systems for PCR reduce tech time, deliver faster and reliable results, minimize handling and processing errors, and help improve confidence in reporting patient results. For these reasons, the cobas® 4800 System was developed.

Objective: To review the key design features and testing solutions of an innovative molecular diagnostic platform which address the automation and integration challenges faced by molecular diagnostic laboratories.

Methodology: The cobas® 4800 System is an automated PCR system, which offers consolidation of Women's Health, Oncology and Microbiology* testing on a single platform. The system is configured with two units - the cobas x 480 instrument for sample preparation/PCR set up and the cobas z 480 analyzer for amplification and detection. On the cobas x 480 instrument, multiple primary and secondary sample types can be loaded and automatically scanned to allow for positive sample ID tracking. Pipetting channels have built-in liquid-level detection and monitoring of all pipetting steps to ensure quality sample processing. A robotic hand transfers specimens to multiple incubation positions to assist with lysis, washing and elution of nucleic acid. The cobas® 4800 system has built-in enzymatic and engineered contamination controls to prevent sample-to-sample or run-to-run carryover contamination. All processes are controlled by intuitive software, which guides the operator through initiating a run, monitoring of the instrument and run status. To increase efficiency a new run can be started in parallel with the amplification and detection of a previous run. User defined workflow software provides open-mode capabilities on the cobas z 480 allowing the laboratory to design customized applications that fit their needs, offering the potential for further platform consolidation.

Results and Conclusions: The cobas® 4800 System test offerings cover a broad range of disease state biomarkers and a diverse array of specimen types. For instance, testing for high-risk HPV and HPV 16/18 genotyping can be done on cervical specimens collected in PreserCyt either before or after cytology processing in order to accommodate sample workflow. *C. trachomatis* and *N. gonorrhoeae* infection can be assessed from male and female urine specimens, endocervical swabs, self-collected or clinician-collected vaginal swabs and cervical specimens collected in PreservCyt solution, which supports CDC recommendations for testing with a wide range of specimen types. BRAF and EGFR mutation testing requires formalin-fixed paraffin embedded tissue sections, including those already mounted on a glass slide. Other tests currently in development include KRAS*, MRSA/SA*, HSV-1/2* and *C. difficile**. Collectively, the cobas® 4800 System is an innovative molecular diagnostic solution that addresses the increasing demand for test integration and sample flexibility.

*The cobas® MRSA/SA Test, cobas® HSV 1 and 2 Test, and the cobas® Cdiff Test are currently in development and not available for sale in the US.

B-075

Metabolomics approach to predict disease severity in influenza virus infection

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Background: Influenza A virus spread on a worldwide scale and infects a large proportion of the human population. Early diagnosis and treatment can have an important role in preventing the development of long-term complications or in interrupting transmission of the infectious agent. A promising approach for the predicting disease progression of influenza infection is targeting host factors that affect disease outcome. Measuring metabolites represents the dynamic metabolomics status of living system. Therefore metabolites levels can be regarded as the ultimate response of biological systems to virus infection. This proposal is using metabolomics strategy to decipher the disease progression after pathogenic influenza A infection.

Methods: Three different strains of influenza A viruses were used to investigate how these different pathogenicity of influenza viral strains affect the metabolism of the host. The three different influenza A H1N1 strains are A/Taiwan/141/2002 (141), A/Taiwan/126/2009 (swine-origin influenza virus, SOIV) and A/PR8/34 (PR8). These three strains have the same antigenicity in their hemaglutinin and neuraminidase (H1N1). PR8 is a high-pathogenicity, SOIV is defined as moderate-pathogenic strain and 141 is defined as mild-pathogenic strain. Female C57Bl/6 animal (6-12 weeks) were anesthetized with Isoflurane and then infected by intranasal application of 200 PFU of viruses. Mice were monitored and weighted daily. Infected and naïve mice (3 mice per group) were sacrificed on day 7 after infection. Bronchoalveolar lavage fluid (BALF) samples were collected and apply to liquid chromatography MS/MS assay based metabolomic analysis (AbsoluteIDQTM180 kit). Dissected mouse lung were fixed and stained with hematoxylin and eosin for pathologic evaluation.

Results: Animals infected with PR8 had 23% weight loss and heavy leukocyte infiltration was observed in lung. Principal component analysis was used to analyze the correlations between the metabolites concentration in samples obtained from naïve mice and mice after different strains of influenza infection. It shows significant difference in these four groups of BALF samples. All the amino acid concentrations were dramatically elevated in PR8 infected mice reflected the extremely active immune response. Long chain acylcarnitine were accumulated when the mice was infected with PR8. Short chain acylcarnitines help the body produce energy and help increase circulation. Acetylcarnitine (C2) was thought to be a more bioavailable form for cells and can induce weight loss. It was significant elevated in PR8 infected BALF. Sphingomyelins were significantly increased in 141 infected BALF; there was no significant difference between the naïve and the SOIV and PR8 infected groups.

Conclusion: Amino acids concentrations in mice BALF are correlated with the

severity of influenza infection. Combination of multiple markers of amino acids, acylcarnitines, sphingomyelines can help to predict the severity of the infection. The metabolic profiling could be a useful method applied to diagnose patients with H1N1infection and can predict the disease severity.

B-076

Evaluation of the Dynex M² MMRV Multiplex Immunoassay Panel vs. Three Commercial Test Kits

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Background: Multiplex analysis of clinical samples offers significant advantages in terms of sample usage and processing time but acceptance has been hindered by high initial costs, lack of full automation, or both. We have developed M²®, a robust, fully automated and cost effective chemiluminescent multiplex immunoassay system to address these shortcomings. Here we present performance comparisons of the M² multiplex panel vs. two qualitative singleplex and one multiplex immunoassay system for antibodies against Measles, Mumps, Rubella and Varicella-Zoster Virus (MMRV).

Methods: Polystyrene beads separately coated with antigen to MMRV immobilized within 96-well M² assay strips. Positive and negative control beads were coated with goat anti-human IgG and combined MRC-5 and E6 cell lysate. Samples were 32 previously characterized human plasma and 7-point 4-fold dilution series of a 5-donor pool of normal serum and run on a modified Dynex DS2® automated ELISA processing system. Identical samples were processed using conventional ELISA kits and a commercially available multiplex assay designed for the Luminex® Model 200.

Results: Sensitivity and specificity of the M² system was calculated independently for each kit using the 32-member reference panel. For each commercial system any sample that tested above the negative cut off was considered as a True Positive (TP), and any sample that fell below was considered a True Negative (TN). Sensitivity was calculated as TP/(TP+FN). Specificity was calculated as TN/(TN+FP). Dilution series of pooled positive serum shows M² to possess greater assay-assay reproducibility than any of the three kits that were examined.

Conclusion: The Dynex M² multiplex immunoassay system shows excellent correlation in both sensitivity and specificity vs. commercial ELISA and multiplex kits across all analytes in an MMRV panel.

Sensitivity and Specificity of Dynex M² vs. Commercial Kits

	Predicate System	Measles	Mumps	Rubella	Varicella
Sensitivity, %	SinglePlex 1	100.0	89.1	100.0	100.0
	SinglePlex 2	85.0	89.1	90.0	100.0
	Luminex	87.5	89.1	82.0	95.5
Specificity, %	SinglePlex 1	100.0	100.0	100.0	100.0
	SinglePlex 2	100.0	100.0	100.0	92.3
	Luminex	100.0	100.0	100.0	100.0

B-077

Workflow Efficiency Through the Use of Mixed Batch Testing for Microbiology Applications on the cobas® 4800 system

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Introduction: System flexibility for molecular diagnostic testing is becoming increasingly important for clinical laboratories as space constraints and staffing shortages impact efficiency. The ability to simultaneously run multiple assays on a single instrument can reduce turn-around time, improve workflow, and have a positive impact on job satisfaction for laboratory technologists. Mixed batch testing streamlines laboratory processing through the use of automated sample extraction and amplification with identical parameters optimized for multiple applications, allowing users to mix samples and tests being included in a single run on the same instrument system. The purpose of this study was to determine the impact of mixed batch testing for *Clostridium difficile* (Cdiff), Methicillin-resistant *Staphylococcus aureus* (MRSA), and Herpes simplex virus (HSV) when evaluated on the cobas® 4800 system compared with 3 other configurations of commercially available systems.

Methods: Batches of specimens for MRSA, Cdiff, and HSV were tested with molecular diagnostic systems in the most efficient possible configuration; system A - BDmax™ Cdiff and MRSA and BD Viper HSV, system B - GeneXpert® XVI Cdiff and MRSA, and BD Viper HSV, system C - GeneXpert® Infinity 48 Cdiff and MRSA, and BD Viper HSV. Batch sizes of 46, 22, and 6 (including controls) MRSA, Cdiff, and HSVspecimens, respectively, were assessed with the cobas® MRSA/SA Test*, cobas® Cdiff Test*, and cobas® HSV 1 and 2 Test* to reflect sample numbers that would be processed on a typical day in a medium sized clinical laboratory. Hands-on time is defined as the labor elements associated with each system/process required to start and finish a testing run that require a manual interaction. Automation time is defined as the time during testing where the operator has no manual interactions with the samples. Turn-around time is the actual clock time from start to finish to complete a testing cycle.

Results: Mixed batch testing on the cobas® 4800 system showed improvement for hands on time of 3, 5, and 6-fold less than comparator systems A, B, and C, respectively, when processing the same number of specimens. Automation time for comparator systems A, B, and C was 2.5, 2.4, and 2.2 fold higher than what was observed with the cobas® 4800 system running mixed batch testing. Evaluation of each configuration showed mixed batch testing improved workflow by reducing turn-around time by 70%, 39%, and 33% over method A, B, and C, respectively.

Conclusions: The system flexibility the cobas® 4800 system allows for mixed batch sample testing for MRSA*, C.diff* and HSV* on a single system which can provide superior workflow efficiency for the increasing demands of the clinical laboratory.

* The cobas® MRSA/SA Test, cobas® HSV 1 and 2 Test, cobas® Cdiff Test and the cobas® KRAS Test are currently in development and not available for sale in the US

B-078

Next-Generation Sequencing for Hepatitis B Genotype and Resistance Testing in a Clinical Microbiology Laboratory

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Objective: Comparison of hepatitis B (HBV) genotyping and resistance testing utilizing an in-house developed PCR assay and next-generation sequencing with a line-probe assay.

Introduction: HBV is one of the most common causes of cirrhosis and hepatocellular carcinoma worldwide. Antiviral therapy has been associated with a delay in disease progression. In addition, HBV genotypes have been associated with different rates of development to advanced liver disease and responses to interferon based therapy. Newer diagnostic modalities such as next-generation sequencing (NGS) can provide both genotype and resistance testing in one assay, and have the potential for increased sensitivity and detection of resistant HBV subpopulations.

Methods: 80 clinical patient samples (plasma) were retrospectively studied. Genotype (n=50) and resistance (n=80) were previously characterized by line-probe assay (INNO-LiPA HBV DR Assay, Version 2/3 and INNO-LiPA HBV Genotyping Assay; Innogenetics, Gent, Belgium). An in-house developed assay for hepatitis B genotype and resistance testing was studied using the GS Junior (454 Life Sciences, Branford, Connecticut). DNA was extracted using the MagNA Pure LC 2.0 (Roche Diagnostics, Mannheim, Germany). PCR amplified a 418bp amplicon of the polymerase region (codons 143 - 281). Amplicons were then sequenced on the GS Junior following the manufacturer's protocols. A third party bioinformatics software company (ABL TherapyEdge, Luxembourg) provided support in the interpretation of genotype and resistance profile of the HBV based on EASL Clinical Practice Guidelines. Sanger sequencing of the PCR amplicons was performed using the 3730 DNA Analyzer (Applied Biosystems, Foster City, USA) for discrepant genotype results between the line-probe assay and NGS.

Results: 50 samples were compared to the INNO-LiPA HBV Genotyping. There was concordance in 47/50 samples (A=3,B=24,C=14,D=5,E=1). Sanger sequencing for the 3 discrepant samples (NGS = B,C,C vs. INNO-LiPA=E,D,B, respectively) confirmed the results of the next-generation sequencing assay. Resistance testing for 80 samples included mutations at the following loci: M204V/I, L180M, A181T/V, N236T, V173L, T184G and S202I/G. No resistance mutations were detected by line-probe assay in 61 samples. Five of these samples had a mutant subpopulation (% of the virus population with a base pair mutation at known resistant loci) detected by NGS: 2 samples with M204I (1.6%; 2.5%), 1 sample with M204I(100%)/L180M(2.5%), 1 sample with V173L (2.5%) and 1 sample with A181T (3.9%). 19 samples with resistance mutations detected by line-probe were also confirmed by NGS.

Conclusions: Utilizing an in-house developed assay with a novel PCR targeting the polymerase region of HBV, genotyping and resistance testing for the most significant mutations can be performed with 1 PCR and 1 NGS reaction. NGS can potentially provide clinicians with increased sensitivity, earlier detection and detailed analysis of resistance profiles, as well as accurate detection of genotype. As a result, next-generation sequencing may become more accessible to incorporate into clinical microbiology laboratories for hepatitis B genotyping and resistance testing.

B-079

An Immunoturbidimetric Assay for Hyaluronic Acid

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Background: Hyaluronic acid (HA), also known as hyaluronate or hyaluronan, is a linear glycosaminoglycan - a high molecular weight polysaccharide with an unbranched backbone composed of alternating sequences of β-(1-4)-*D*-glucoronic acid and β -(1- 3)-*N*-*D*-acetylglucosamine moieties. Each dimer is referred to as one unit and has a molecular weight of approximately 450 Da. The HA molecule can vary in length from less than 10 to more than 1,000 units. HA is mainly produced by fibroblasts and other specialized connective tissue cells. It is a major constituent of connective tissue matrix (proteoglycan) and participates in various cell-to-cell interactions. HA is widely distributed throughout the body and can be found as a free molecule in plasma and synovial fluid. In plasma, the half-life of the HA molecule has been estimated to be about 5-6 minutes. HA is found in synovial fluid in high concentrations and is responsible for normal water retention and articular lubricant. Synovial HA may pass into plasma via the lymphatic system. In circulation, HA levels are maintained by an efficient receptor-dependent removal mechanism present in sinusoidal endothelial cells (SEC) of the liver and by the enzymatic action of hyaluronidase. Because the liver plays a central role in maintaining HA homeostasis, increased plasma levels of HA may serve as a sentinel for hepatic inflammation, fibrosis and cirrhosis. We report here the development of an immunoturbidimetric method for detecting HA in a blood sample.

Methods: An R1 reagent/reaction buffer was developed and optimized to augment the specific agglutination reaction of the coated microparticles with hyaluronic acid in the serum samples. An R2 reagent/coated polystyrene microparticles was developed using functionalized polystyrene microparticles covalently coated with HA binding proteins using standard conjugation techniques. Iterative combinations of R1 and R2 reagents were systematically tested to achieve consistent linearity and precision. Numerous iterations of coating and blocking buffers were assayed to further enhance assay manufacturability and consistency. Finally, in-process testing of linearity and precision was conducted to ensure robust performance to the end-user.

Results: The assay's limit of detection (LOD) was determined to be 11.36ng/mL; limit of blank (LOB) was found to be 6.68ng/mL; limit of quantitation (LOQ) was determined to be 20.00ng.mL. Rigorous precision testing demonstrated the assay's consistency of the course of 20 operating days with a 5.3% overall percent coefficient of variation. Assay linearity was between 25ng/mL to 750ng/mL for samples tested. The overall average percent recovery was 103.5% and lot to lot values showed no statistical difference ($p= 0.736$) across both the medical decision range and the range above. Real time stability concluded the assay can reliably and consistently measure samples over the course of at least 12 months, with a deviation of less then 10% of the mean for each group.

Conclusions: The data presented herein highlight the robust performance of this immunoturbidimetric assay. In summary, these data demonstrate the overall performance of the assay was consistent with a predicate HA-ELISA and that values obtained will be consistent both over time and from lot to lot.

B-080

PCR-Reverse Blot Hybridization Assay for Identification of Pathogens causing Sepsis from Positive Blood Cultures

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Background : Sepsis is a lethal medical condition leading to the systemic inflammatory response to infection. Sepsis is the 10th leading cause of death in the United States, accounting for 6% of all deaths and an estimated 135,000 patients die each year of sepsis-associated complications in Europe. Early detection of pathogens responsible for septicemia and antimicrobial resistance are significantly important for appropriate antimicrobial therapy. This study aimed to evaluate the PCR-reverse blot hybridization assay (PCR-REBA) capable of the identification of pathogens and antimicrobial susceptibility test (AST) in blood culture specimens.

Methods : The PCR- REBA, REBA Sepsis-ID® (M&D, Wonju, Republic of Korea) was designed to contain a total of 25 probes including 6 Gram-positive bacteria (GP) specific probes, 8 Gram-negative bacteria (GN) specific probes and 5 *Candida* species specific probes with a pan-bacteria, a pan-GP and a pan-GN probes. In addition, it includes *mec*A ,*van*A and *van*B probes for detection of antibiotic-resistant bacteria. For evaluation of the REBA Sepsis-ID®, a total of 300 positive blood culture bottles from BACTEC™ FX (Becton Dickinson Diagnostic System, Spark, MD, USA) or BacT/ALERT 3D (bioMérieux, Marcy, France) were used. The identification of organisms and antimicrobial susceptibility tests (ASTs) were conducted by the microplate method, the MicroScan® system (Siemens Healthcare Diagnostics, Sacramento, CA, USA), and the Vitek® 2 system (bioMérieux).

Results : The correct agreement rates between conventional identification and AST methods and PCR-REBA for GP, GN, *Candida* and polymicrobials were 94.5%, 97.3%, 100% and 91.7%, respectively. Of 92 methicillin-resistant *Staphylococcus* species, *mec*A gene was detected in 90 (97.8%) samples and *van*A gene was correctly detected in one (100%) sample which was identified to vancomycin-resistant *Enterococcus* (VRE) by phenotypic examination.

Conclusion : Newly developed REBA Sepsis® was a rapid and accurate molecular-based method for simultaneous rapid detection of causative agents and antimicrobial resistant genes in positive blood cultures even though there was a limitation for evaluation with negative cultures such as nonviable after exposure to antibiotics or small amount bacteria.

B-081

Assessment of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with the cobas® CT/NG v2.0 Test on the cobas® 4800 system: Infection prevalence in pregnant women enrolled in a large multicenter clinical trial

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Background: Miscarriage, pre-term delivery, low birth weight, and morbidity in the neonate are potential consequences when pregnant women become infected with sexually transmitted diseases. In an effort to identify women with infection, treatment guidelines recommend screening pregnant women for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhea* (NG) on the first prenatal visit. This study was performed to determine the frequency of CT and NG infection observed in pregnant women enrolled in a large clinical trial study population.

Methods: This multicenter retrospective cohort analysis was performed with archived specimens collected during the VENUS clinical trial and prospective specimens collected during the VENUS II clinical trial, to characterize the clinical performance of the cobas® CT/NG v2.0 Test on the cobas® 4800 system. As recommended by the FDA, Patient Infected Status (PIS) was determined for each enrolled participant using two FDA-cleared nucleic acid amplification tests (NAATs) as comparator assays. PIS was defined as positive when results from NAATs with different target regions generated positive results with collected samples. Diverse settings in the United States served as specimen collection sites and included obstetrics-gynecology practices, family planning clinics, and STD clinics.

Results: Of 6,035 eligible participants, 6,004 subjects were evaluated for CT and/or NG for primary anlaysis. PIS determined 365 women and 92 men were infected with CT, and 122 women and 67 men were infected with NG. Of the female patients evaluated, 6.93% (365/5265) were found to be positive for CT infection and 1.75% (92/5265)were positive for NG according to PIS outcomes. Alternatively, 8.4% (17/202) of eligible pregnant women were positive for CT, where 1.48% (3/202) of pregnant women were considered positive for NG by PIS.

Conclusion: Screening of pregnant women for CT and NG with the cobas® CT/NG v2.0 Test on the cobas® 4800 system compared to two additional NAATs during the VENUS clinical trial revealed comparable rates of infection for CT and NG between pregnant women and non-pregnant women in the general female population.

B-082

Performance evaluation of the Access HCV Ab PLUS assay on the UniCel DxI 800 system

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Background: The Access® HCV Ab PLUS assay (Bio-Rad) is a chemiluminescent microparticle immunoassay (CLIA) for the qualitative detection of antibodies to the hepatitis C virus in human serum or plasma. The purpose of these studies was to evaluate diagnostic performance.

Four serological automated assays were compared: Access HCV Ab PLUS assay on UniCel® DxI 800 Immunoassay system (Beckman Coulter Inc.), Architect® anti-HCV assay on Architect I2000SR analyser connected to the APS system (Abbott Diagnostics), Elecsys® Anti-HCV II assay on MODULAR® ANALYTICS E170 system or Cobas® e601 system (Roche Diagnostics) and ADVIA Centaur® HCV assay on ADVIA Centaur XP system (Siemens).

Methods: The Access HCV Ab PLUS assay is a two-step indirect antibody detection format.

First study: 659 fresh samples tested for HCV diagnosis in the routine virology laboratory at the University hospital of Angers were prospectively tested, 199 frozen positive samples from a retrospective data collection of patients' sera and 2 commercial panels were tested on Access HCV Ab PLUS and Architect anti-HCV assays. Two other commercial panels were tested only on Access assay. For Architect assay, data from the supplier were used.

Second study: the specificity was estimated by testing 500 non-selected fresh serum samples from a routine laboratory, 3 commercial panels plus one anti-HCV low titer performance panel were tested on Access HCV Ab PLUS, Elecsys anti-HCV II and ADVIA Centaur HCV assays. Another panel was used on Access and Elecsys assays. For ADVIA Centaur assay, data from the supplier were used.

Results: First study: On unselected routine samples, the agreement between the two assays was equal to 99.5%. The clinical specificity was 98.9% (95% CI: 97.8-99.6%) and 99.7% (95% CI: 98.9-100%) for Access HCV Ab PLUS and Architect anti-HCV assays, respectively. The clinical sensitivity for all positive samples was 100% for both assays. The sensitivity on seroconversion samples showed performance in accordance with the state-of-the-art for Access and Architect assays.

Second study: 488 of the 500 non-selected samples were true negative. The clinical specificity was 99.6% (95% CI: 98.53-99.95%) for Access HCV Ab PLUS, Elecsys anti-HCV II and ADVIA Centaur HCV assays. The concordance between the 3 assays was 99.20%. The clinical sensitivity from 12 positive samples was 100% for all assays. Using 4 commercial seroconversion panels and one anti-HCV low titer performance panel, Access HCV Ab PLUS and Elecsys anti-HCV II assays showed equivalent performance and detected earlier than ADVIA Centaur HCV assay.

Conclusion: The performance of the Access HCV Ab PLUS assay on the UniCel DxI 800 immunoassay system was excellent in terms of specificity and sensitivity. The clinical specificity was slightly better with Architect anti-HCV assay as compared to the other assays. The clinical sensitivity on true positive samples was 100% for all assays. The seroconversion sensitivity was better on UniCel DxI 800, Architect and Modular than on ADVIA Centaur. Adapted for high throughput routine testing, the Access HCV Ab PLUS assay performed on UniCel DxI 800 immunoassay system is fully suited for the screening of HCV infection in diagnostic laboratories.

B-084

Carbapenem-Resistant Enterbacteriaceae (CRE) in Geriatric Population:

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Background: CRE refers to Carbapenem-Resistant and/or Carbapenemase-producing Enterobacteriaceae. These are families of bacteria that are resistant to several classes of antibiotics including one of the carbapenen group. Carbapenem antibiotics are used to treat infection caused by gram negative bacteria as the last line of treatment. Multidrug-resistant gram negative bacteria especially CRE are becoming the new super bug in the Long-Term Care Facilities where most of the residents are elderly, frail and are on multiple medications. The most common enterobacteriacae are *Klebsiella* species and *Escherichia col.;* over 40% mortality has been reported with invasive infection with CRE. Infection with CRE has limited therapeutic and high morbidity and mortality.

Methodology: 35,330 specimens were collected for culture from residents in Long-Term Care Facilities over a period of 6 months. All positive cultures were subcultured and then identified using MicroScan Walkaway 96 conventional panels, the isolate was considered CRE if it was resistant to one or more of the carbapenem, with Ertapenem nonsusceptibility being the most sensitive indicator of carbapenemase production. Statistical analysis were done using Analyse-it.

Results: 18,569 (52.6%) specimens were positive, 320 patients had CRE positive culture, the most common source was urine 250 cases (78.1%) followed by wound 42 cases (13.1%), respiratory 16 cases (5.0%)), rectal swab 5 cases (1.6%) and blood 4 cases (1.3%). Majority of the cases were reported in August (23.8 % of all cases) and the lowest was in November (11.3 % of all cases); we noticed an increase in the cases in January which was due to respiratory infections. The most common organism was *Klebsiella Pneumoniae (ESBL or MDR),* followed by *E. CLOACAE MDR, E. Coli,* and *SERRATIA MARCESCENS.*

Conclusion: CRE incidence is high in the long-term care facilities, facilities should follow the CDC recommendation to implement the "detect and protect" strategies. Prompt implementation of infection prevention and control measures requires close collaboration between clinical laboratory, infection prevention staff, physicians, and nurses. Early detection and implementing infection control and prevention will help reducing the transmission to other residents in addition to identifying the risk factors for CRE. Cautious and appropriate use of antimicrobial therapy for the treatment of suspected infections in residents of long-term care facilities are very important to prevent the occurrences.

B-086

Reduced Methicillin-resistant *Staphylococcus aureus* infections rate after the three-year implementation of a Rapid Molecular Screening in Intensive Care Unit

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Background: Previous studies have suggested that Methicillin-resistant *Staphylococcus aureus* (MRSA)-colonized patients are at higher risk for acquiring MRSA infections compared with non-colonized patients, while in the hospital, thus, MRSA-carriers should be monitored closely. Molecular screening methods have the advantage of high sensitivity and rapid turnaround times (TATs), assuring a rapid delivery of results and, consequently, improving infection control procedures and the clinical impact. Here, we assess the effects of implementation of a specific MRSA bundle, based on rapid molecular screening for MRSA and decolonization, on the prevalence of MRSA infection at our ICU, over a study period of three years.

Methods: The study was conducted over two time periods, before and after implementation of a specific MRSA bundle. A total of 431 and 886 nasal screening swabs were obtained from ICU patients, respectively before and after the bundle implementation and analyzed by the molecular test Xpert® MRSA (Cepheid). The pre-bundle period (from April 2009 through December 2010), has been used to assess the rate of ICU colonization and to evaluate the more appropriate measures to be applied in MRSA-carriers, thus screening results did not activated any preventive measures in patients colonized. Later, an MRSA bundle was implemented from January 2011 through December 2013 at our ICU (post-bundle period). The bundle consisted of rapid molecular screening for MRSA nasal carriage, at the ICU admission, contact precautions and nasal decolonization (mupirocin 2% ointment three times-a-day for five days) for patients colonized with MRSA. Clinical samples from patients suspected as being infected with MRSA were tested by standard laboratory culture procedures. The results of rapid nasal screening were available to physicians within 2 hours from specimen receipt.

Results: In the pre-bundle period, 9 patients (2%) developed a generalized MRSA infection, but during the three years that followed the bundle implementation (post-bundle period), MRSA infection rates declined from 2 % to 0.2% (2 patients) with a total MRSA infection decrease of 100% in the third year post-intervention. On the contrary, MRSA colonization rates at admission increased from 7,1% in the pre-bundle period to 8,6 % in the post-bundle period. Overall, during the three years post-intervention, the relative risk reduction, absolute risk reduction and relative risk were as follows: 0.9 (95% confidence interval: 0.58-0.98), 0.26 (95% confidence interval: 0.1-0.33) and 0.09 (95% confidence interval: 0.014-0.4), respectively. Moreover, the risk of MRSA infections among colonized patients, already reduced in 2011 (Relative Risk 0.18, 95% confidence interval: 0.008-1.1) and 2012 (Relative Risk 0.12, 95% confidence interval: 0.006-0.8), compared with the pre-bundle period, dropped

dramatically in 2013 (Relative Risk 0.000, 95% confidence interval: 0.000-0.6), with no case of MRSA infection reported.

Conclusion: The present study showed that a strategy of active surveillance based on rapid molecular screening for MRSA, immediately after admission, rapid reporting and prompt nasal decolonization, resulted in a significant decline in MRSA infections rate in our ICU over the three years post-bundle period. Real time PCR demonstrated a superior sensitivity to culture and rapid TATs, allowing a better management of MRSA-carriers who will more likely develop MRSA infections.