Wednesday, July 29, 2015

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

Infectious Disease

B-058

Comparison of respiratory viral panel detection by Mediator probe and hydrolysis probe PCR

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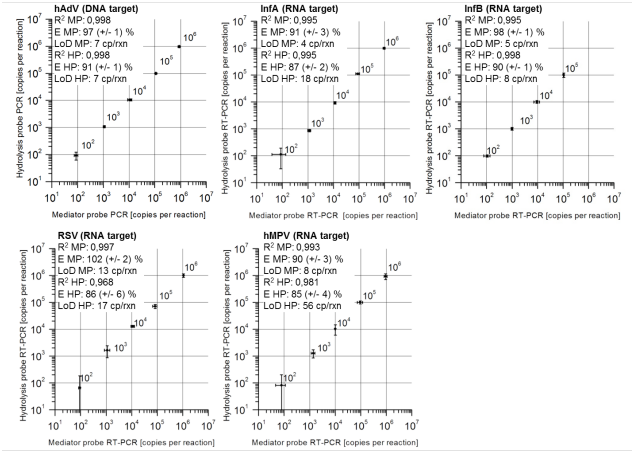
Background: Molecular diagnostics often uses hydrolysis probes (HP) for real-time nucleic acid sensing. However, each target sequence requires synthesis of specific dual-labelled HPs, which are expensive, especially when used at low batch sizes. Also, HPs must be individually optimized for signal generation efficiencies for each target sequence to be detected. We have published a novel approach, the mediator probe PCR (MP PCR) [B. Faltin et al.: Clin Chem (58) 2012] which overcomes these issues by using a labelled but universal reporter oligonucleotide (UR) as a biosensor for target-independent signal generation. It is triggered by unlabelled and thus cost-effective sequence-specific mediator probes. Compared to [Faltin 2012] we improved UR quenching efficiencies and reaction setup of MP PCRs to detect 5 different DNA and also RNA target sequences of viruses causing respiratory tract infections. HP based assays, which required 5 different dual-labelled probes were run as references.

Methods: MPs and the UR designs were adapted from [Faltin 2012] with the sequence-specific MP section equal to corresponding HP sequences. Nucleic acid standards from human adenovirus (hAdV), influenza virus A&B (InfA & B), human metapneumovirus (hMPV), and respiratory syncytial virus (RSV) were serially diluted enabling efficiency calculation and detection limit determination.

Results: Reaction efficiencies (E), correlation of DNA/RNA input and back-calculated output concentrations and the limit of detection with 95 % probability (LoD) were:



They correspond well to commercially available assays [L. Van Wesenbeeck et al.: J. Clin Microbiol. (51) 2013].

Conclusion: One UR was used for sensing 5 different DNA and RNA targets by MP (RT-) PCR. Even higher reaction efficiencies and lower detection limits as with the more expensive HP (RT-) PCRs could be reached. The method is especially recommended if many different target-specific probes are required at low batch sizes. In future, multiplexing degrees shall be increased using UR-microarrays.

B-059

Laboratory values from positive EBOV patients

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Background: The ongoing epidemic of Ebola Zaire (EBOV) in West Africa has resulted in a handful of patients with the disease to be treated in Western medical facilities. With these opportunities, more about EBOV disease course and treatment has been discovered. There is no published literature investigating routine laboratory values for individuals with EBOV.

Methods: Four patients with confirmed EBOV were admitted to the specialized isolation unit at Emory University between August and October 2014. All laboratory values were generated within the dedicated isolation laboratory using the following instruments: Chemistry analyzer (Abaxis Piccolo Xpress [ABAXIS, Inc, Union City, CA]), Blood-gas analyzer (GEM Premier 4000+[Werfen, Barcelona, Spain]), Automated urinalysis analyzer (CLINITEK Status [Siemens Corp., Munich, Germany)], and Hematology analyzer (pocH 100i [Sysmex Corporation, Kobe, Japan]). Testing was done daily in most instances; frequency increased and decreased depending on the status of the patient and the needs of the medical team.

Results: The patients presented upon admission with varying degrees of hypoproteinemia, hypocalcemia, elevated liver enzymes, and some electrolyte abnormalities. In the two patients with complete blood cell count results (CBC) on admission, platelet levels were mildly suppressed. Some, but not all of the laboratory values normalized by discharge. Notably liver enzymes were still elevated in three patients. Calcium, albumin, and total protein levels remained suppressed throughout most of the patients' recovery. All four patients showed decreased platelet counts during their hospitalization. Aspartate aminotransferase and alanine aminotransferase levels were significantly elevated (500- 1000 U/L) in two of the four patients. Alkaline phosphatase was elevated in three of the four patients. Red blood cell count (RBC) and hemoglobin levels fell during the course of disease in all four patients, with no evidence of gross hemorrhage, and these values remained low at discharge for all 4 patients. One patient with severe acute kidney injury had consistently elevated BUN and creatinine levels throughout their treatment.

Conclusion: The patients varied in symptom severity which was reflected in the laboratory values. Also, the patient with the least severe course of illness had more normal laboratory results except for the liver enzymes which were among the highest during hospitalization. These laboratory values are typical of patients dealing with acute viral infections and would be predicted in conditions associated with hemorrhage.

B-060

Evaluation of a Multiplex Array for the Simultaneous Detection of Ten Common STI Pathogens

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Background: Sexually transmitted infections (STIs) present a major public health concern worldwide with more than 1 million people acquiring an STI every day. Timely access to testing and treatment services can reduce the risk of onward transmission, however many STIs are asymptomatic and some display similar or overlapping symptoms, thus co-infections may remain undiagnosed. Minimum tests recommended in British Association for Sexual Health and HIV (BASHH) guidelines, include chlamydia, gonorrhoea and syphilis (*Treponema pallidum*); symptomatic women may also be tested for *Trichomonas vaginalis,* which causes vaginitis and

cervicitis in women and urethritis in men. *Trichomonas* infections are often asymptomatic and not tested for because prevalence is assumed to be too

low. In addition, wet mount microscopy, the routine diagnostic method for women, is insensitive and therefore *T.vaginalis* infection remains

underdiagnosed. New BASHH guidelines now recommend Nucleic Acid Amplification Tests (NAAT) for *T.vaginalis*. HSV testing is offered to both men and women in the presence of genital ulceration. Mycoplasmas and Ureaplasmas are not routinely tested for although *M. genitalium* is now accepted as an STI, being implicated in urethritis and cervicitis, but the previous lack of recognition has lead to inappropriate treatments and a significant rise in antimicrobial resistance. *U.*

urealyticum has also been associated with recurrent urethritis and, along with *M. hominis* and *M. genitalium* with some cases of pelvic inflammatory disease (PID).

In this context, the need for efficient means of detecting these infections has become increasingly important. This study reports the evaluation of a multiplex array on a biochip platform for simultaneous detection of ten common STI pathogens from a single sample. This approach increases detection capacity, with the potential of identifying more asymptomatic infections and co-infections.

Methods: Residual DNA extracted from a urine and urogenital swab anonymous sample cohort (n=869), obtained blind from the Regional Virology Laboratory (RVL, Belfast Health and Social Care Trust), were tested for the presence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), Herpes simplex 1 and 2 (HSV1, HSV2), *Treponema pallidum* (TP), *Trichomonas vaginalis* (TV), *Haemophilus ducreyi* (HD), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH) and *Ureaplasma urealyticum* (UU) using the STI Multiplex Array on the Evidence Investigator analyser (Randox Laboratories Limited, Crumlin, UK). The protocol involves amplification of DNA using highly sensitive primers, followed by spatial separation and detection using biochip array technology. Assay results were compared against the RVL sample diagnosis and discrepant samples re-tested.

Results: Agreement with predicate assay(qPCR) was ≥94%. Analitical sensitivity was 100%, specificity ranged from 94% to 100% for all key targets. Of the 869 samples, 66% were negative, 27% positive for one infection and 7% harboured at least one additional infection. Results were confirmed by uniplex real-time PCR or another commercial assay.

Conclusion: The data indicate that this multiplex array assay detects simultaneously 10 common STI pathogens from a single sample without compromising sensitivity or specificity, furthermore it facilitates the identification of co-infections. This leads to increased diagnostic capabilities, which may allow tailored treatment, reducing broad spectrum antibiotic use and, in turn, the build-up of antibiotic resistance.

B-061

Detecting Acute HIV in a High Incidence Setting - Los Angeles and 4th Generation HIV Ag/Ab Testing

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Background:

In its 2013 report, the CDC tracked the rate of new HIV infections per capita (2011 data). Out of the top 25 U.S. cities with the highest rates, Los Angeles ranked 19th. This rate marks a need for the earliest possible detection mechanisms in such high prevalence areas. Historically, ~37% of individuals who are eventually diagnosed with HIV progress to AIDS within 6 months indicating that many are not caught before acute symptoms fade and do not seek testing until symptoms return in late stages.

During the acute stage, patients do not develop measurable antibodies to HIV. Consequently, testing these patients with third generation tests, which only detect antibodies, will yield a negative result. Only the 4th Generation HIV antigen antibody (Ag/Ab) Combo test and nucleic acid amplification tests (PCR/NAAT) are able to detect acute HIV infections.

Methods and Results:

Over a 15-month period at Cedars-Sinai Medical Center, 7 acute HIV-1 cases were detected by the Abbott ARCHITECT 4th Generation HIV Ag/Ab Combo test. An acute diagnosis constituted Combo positive, Western blot negative results, which were confirmed positive by PCR. Of the 7 acute cases, 6 presented to the ED with acute symptoms. Additionally, the false positive rate for the ARCHITECT Combo test was calculated at 0.08% (11 cases) for 12388 patients tested in the selected time period; Furthermore, the Combo assay detected 0.11% more cases (8 cases) than our previous BioRad Genetic Systems 3rd generation immunoassay.

Conclusion:

These cases demonstrate that it is imperative for first line HIV testing to be capable of detecting both acute and established HIV infection with accuracy, particularly in high transmission areas. By stopping new infections from occurring through early and accurate diagnosis, we can approach a future where rampant transmission becomes a thing of the past and the number of people living with HIV becomes relatively static.

7 acute HIV cases detected with 4th Generation Combo Assay at Cedars-Sinai (15 month timeframe)

Case	Patient	Presentation/Symptoms	Abbott ARCHITECT HIV Combo (4th Gen)	Western Blot	PCR Viral Load (copies/mL)
1	34 y/o Male	Presented to ED with fever, headache, chills	POSITIVE	NEGATIVE	156,000
2	34 y/o Male	Presented to ED with fever, rash, enlarged tonsils	POSITIVE	NEGATIVE	3,510,000
3	27 y/o Male	Presented to ED w/ fever/chills	POSITIVE	INDETERMI-NATE	5,600,000
4	49 y/o Male	Outpatient specimen (no further information)	POSITIVE	NEGATIVE	>10,000,000
5	37 y/o Female	Presented to ED with abdominal pain, diarrhea	POSITIVE	NOT TESTED	>10,000,000
6	25 y/o Male	Presented to ED with fever, muscle aches, and diarrhea	POSITIVE	NEGATIVE	1,760,000
7	52 y/o Male	Presented to ED with abdominal pain	POSITIVE	NEGATIVE	3,050,000
	y/o = year old				

B-062

A Novel Immunoassay that Distinguishes between Bacterial and Viral Infections Based on a Patient's Immune Response

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OBJECTIVES: Bacterial and viral infections are often clinically indistinguishable, leading to inappropriate patient management and antibiotic misuse. Effective use of infectious disease diagnostics has been hindered by long waits for results, high costs, inaccessible (or unknown) sites of infection, and the presence of non-disease causing colonizing bacteria that can lead to false positive results. An approach that has the potential to address these challenges relies on monitoring the host's immune-response to infection, rather than direct pathogen detection. Our goal was to develop and validate a novel assay that combines blood borne bacterial- and viral-induced host-proteins that can accurately distinguish between bacterial and viral infections. **METHODS:** We prospectively recruited 1002 hospitalized and emergency department patients with acute infection, and controls with no apparent infection (NCT01917461). For each patient, three independent physicians assigned a diagnosis based on comprehensive clinical and laboratory investigation that included PCR for 21 common pathogens. We quantitatively screened 600 circulating host-proteins and developed a multi-parametric signature using logistic-regression on half of the patients, and validated it on the remaining half. **RESULTS:** The cohort included 319 bacterial, 334 viral, 112 control and 98 indeterminate patients (139 were excluded based on pre-determined criteria). The cohort was balanced with respect to gender (47% females, 53% males) and included 56% pediatric patients (≤18 years) and 44% adults (>18 years). The best performing host-protein was TNF-related apoptosis-inducing ligand (TRAIL) (area under the ROC curve [AUC] of 0.89; 95% confidence interval [CI], 0.86-0.91), which was consistently up-regulated in viral infected patients. The signature with the highest precision included both viral- and bacterial-induced proteins: TRAIL, Interferon gamma-induced protein-10, and C-reactive protein (AUC of 0.94; 95% CI, 0.92-0.96). The signature outperformed routinely-used clinical parameters, such as white blood cell count (AUC of 0.64±0.04), absolute neutrophil count (AUC of 0.73±0.04), % monocytes (AUC of 0.64±0.04), % lymphocytes (AUC of 0.76±0.04), peak temperature (AUC of 0.51±0.04), pulse (AUC of 0.62±0.04), procalcitonin (AUC of 0.67±0.11), and an algorithm that combines these clinical parameters (AUC of 0.78±0.04). The signature was robust across various physiological systems (respiratory, urinary and systemic), times from symptom onset (0-12 days), and pathogens (56 species), with AUCs between 0.87 and 1.0. Finally, the signature's accuracy was not affected by the presence of potential colonizers and it was able

to provide accurate diagnoses even in cases where the infection site was not known or easily accessible. A kit called ImmunoXpert™ was developed, which measures the proteins in 99 minutes using an ELISA format, and computationally integrates the measurements into the final diagnosis. **CONCLUSIONS:** The present signature combines newly identified viral-induced with traditional bacterial-induced host proteins. It provides valuable information over standard laboratory and clinical parameters, which are routinely used in clinical practice today to facilitate differential diagnosis of infection etiology. Furthermore, assay run time can be further shortened as it is readily translatable to hospital-deployed automated immunoassay machines and point-of-need assay formats. This signature has the potential to significantly improve the management of patients with acute infections and reduce antibiotic misuse.

B-063

Validation and Correlation of VIDAS® IgGII and IgMII Lyme Antibodies vs VIDAS® Lyme IgG/IgM in the Laboratory Evaluation of Lyme Disease: Clinical Implications.

U. L. PRISCO, K. J. PRISCO. *VINEYARD MEDICAL CARE, VINEYARD HAVEN, MA*

Objective: To perform method comparison studies between the VIDAS® Lyme IgM II (LYM) and VIDAS® Lyme IgG II (LYG) assays versus the VIDAS® Lyme IgG/IgM (LYT) combination assay currently available for clinical use. A secondary goal was to determine if utilization of separate measurements of IgM and IgG Lyme antibodies done simultaneously would result in a reduction in Lyme western blot assays needed to determine a serologic diagnosis of Lyme Disease.

Methodology: VIDAS®LYT, LYG, and LYM assays are automated, qualitative, enzyme-linked fluorescent immunoassays (ELFA) for use in the detection of human IgG and IgM antibodies to *Borrelia burgdorferi* in serum or plasma. The LYT assay detects and reports total IgG/IgM antibodies whereas LYG and LYM assays are independent and separately measure and report IgG and IgM antibodies to *Borrelia burgdorferi*. The LYT assay requires 35 minutes to complete and the LYG and LYM require 27 minutes to complete.

Previously frozen serum samples from patients with signs and symptoms of Lyme disease were allowed to thaw to RT. Samples (N=114; 100ul) were analyzed using the LYT, LYG and LYM assays. All positives and/or equivocals in any assay were analyzed via standard IgG and IgM Lyme western blot (LYWB) analyses.

The sensitivities of the VIDAS®LYG, LYM assays versus the respective IgG and IgM LYWB assay results were estimated. The sensitivity of the VIDAS®LYT combination assay was estimated using the results of both IgG and IgM LYWB assays. Fisher's exact test was used to determine whether significant differences in sensitivities existed between the two independent assays and the combination assay.

Results: There was a high correlation between VIDAS® assays in terms of positives and negatives. The sensitivity of the LYG assay against LYWB was 90.4% and the sensitivity of the LYM assay was 73.8%. In comparison, the sensitivity of the LYT combination assay was 42.2%. Sensitivities of each of the two independent assays were significantly higher than the combination assay ($p<0.001$).There was a 23.3% reduction in the number of LYWBs needed to definitively determine Lyme diagnosis when separate measurements of LYG and LYM assays were performed compared to the LYT combination assay.

Conclusions: Per CDC guidance, laboratory diagnostic confirmation of Lyme Disease requires serologic analysis of Lyme IgG and IgM antibodies. Currently available testing in the clinical laboratory utilizes the reporting of total IgG/IgM antibodies followed by IgG and IgM western blot analysis to determine disease stage.

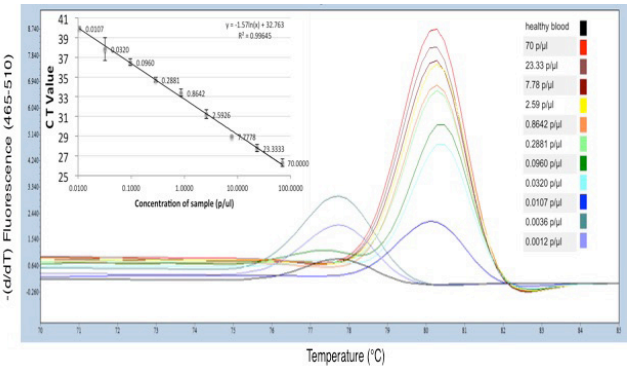
Utilizing the VIDAS® LYG and LYM separately provided enhanced specific laboratory diagnostic information earlier to couple with patient symptomology that was closer to that provided by LYWB. Utilization of separate IgG and IgM assays as first round diagnostic testing provides the ability to quickly separate acute from later stage or historical exposure. Having this information earlier decreases unnecessary and/or inappropriate treatment for previous infections, and provides earlier information aiding differential diagnoses in endemic areas of tick borne disease. The impact on quality of care via reductions in: time to definitive laboratory diagnosis, unnecessary treatments, long-term clinical symptoms and ultimate costs to patients and payors has yet to be determined.

B-064

CLIA-PCR: a High-Throughput PCR Technology for Molecular Screening, with an Application in Malaria Surveillance for Elimination

Z. Cheng[1], X. Sun[2], D. Wang[3], X. Tian[1], Y. Sun[1], N. Xiao[3], Z. Zheng[1]. *[1]Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and School of Basic Medicine, Peking Union Medical College, Beijing, China, [2]Yunnan Institute of Parasitic Diseases, Yunnan, China, [3]National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention, WHO Collaborating Center for Malaria, Schistosomiasis and Filariasis, Key Laboratory of Parasite and Vector Biology, Ministry of Health, Shanghai, China*

Background: sensitive and affordable methods for active screening of malaria parasites in low-transmission settings are urgently needed. Methods: we developed a novel molecular screening technology called Capture and Ligation Amplification PCR (CLIA-PCR), which achieved the sensitivity of reverse-transcription PCR yet eliminating the reliance on RNA purification and reverse transcription. In short, 18S rRNA of genus Plasmodium is released from blood and captured onto 96-well plates, and quantified by the amount of ligated probes that bind continuously to it. We first used laboratory-prepared samples to test the method across a range of parasite densities and pool sizes, then applied the method to an active screening of 3358 dried blood spot samples collected from three low endemic areas in China. Results: Plasmodium falciparum diluted in whole blood lysate could be detected at a concentration as low as 0.01 parasite/µl (Figure), and pool size of up to 35 did not significantly affect the assay performance. When coupled with a matrix pooling strategy, the assay drastically increased the throughput to thousands of samples per run while reducing the assay cost to cents per sample. In the active screening, CLIA-PCR identified 14 infections including 4 asymptomatic ones with less than 500 tests, costing less than 0.6 dollars for each sample. All positive samples were confirmed by standard quantitative PCR. Conclusions: CLIA-PCR, using dried blood spots with a pooling strategy, efficiently offers a highly-sensitive and high-throughput approach to detect asymptomatic submicroscopic infections with dramatically reduced cost and labor, making it an ideal tool for large scale malaria surveillance in elimination settings. More importantly, CLIA-PCR greatly reduced the complexity of PCR test with its ELISA-like workflow, offering the potential to significantly enhance the capacities of molecular laboratories with no need for additional equipment.



B-065

Prospective evaluation of two multiplex real time PCR assays for simultaneous detection of sexually transmitted pathogens

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According to the World Health Organization, sexually transmitted infections (STIs) are transmitted from person to person through sexual contact. It is estimated that worldwide, 448 million new cases of STIs are diagnosed each year. Molecular assays have been described for the detection of pathogens, and multiplex real-time PCR (M-qPCR), which uses multiple primers and probes, is a sensitive, rapid, and high-throughput approach for qualitative analysis of several infectious agents in the same

reaction. This technique has become a mainstay of research and clinical diagnostic applications. In this regard, the widespread prevalence of STIs and the public health costs associated with STIs management have led to the need for fast and reliable methodsfor diagnosing STIs. However, because STIs can be caused by polymicrobial infection methods to identify multiple pathogens in a single sample are necessary. The goal of this study was to develop and optimize a test for the simultaneous detection of five clinically important bacteria associated with STIs, including Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasmahominis, Mycoplasmagenitalium, and Ureaplasmaurealyticum, by Multiplex Real Time PCR (M-qPCR). A total of 10 clinical samples (vaginal swabs) from patients with presumptive diagnosis of infection with these bacteria were examined. Two M-qPCRs were developed: MI: N. gonorrhoeae, C. trachomatis, U. urealyticum; MII: M. hominis, M. genitalium, U. urealyticum. The primers and probes selected in this study were specific for conserved regions of the genes encoding glyceraldehyde 3 phosphate dehydrogenase (GAP/GAPDH) (M. hominis), MgPa surface protein (M. genitalium), urease B subunit (UreB) (U. urealyticum), and outer membrane protein (OMP1) (C. trachomatis), or for unique sequences within the cryptic plasmid (pJD1) (N. gonorrhoeae). The selected primers were consideredadequate and performed satisfactorily in silicoPCR as well as conventional PCR, generating specific products of the appropriate size for each examined infectious agent.All standard plasmids (positive controls) for each multiplex test amplified with Ct value less than 40, including the internal control (β-actin gene), which demonstrated that the extracted genetic material was of good quality. The reactions were able to detect the agents and according to the Ct values and the largest concentration of each point of the standard curve, the following detection limits were determined: 29.7 copies/mL for N. gonorrhoeae, 30.1 copies/mL for U. urealyticum, 29.9 copies/mL for C. trachomatis, 29.7 copies/mL for M. hominis and 30.4 copies/mL for M. genitalium.Among the clinical specimens evaluated, the multiplex reactions resulted in detectable Ct values (below 40) and at least one of the bacterial species was detected.These multiplex Q-PCRs using TaqMan 5´nuclease real-time PCR provided a novel, qualitative method for the rapid detection of diagnosis of STIs associated with N. gonorrhoeae, C. trachomatis, U. urealyticum,M. hominis, and M. genitalium. The results of this study were notably encouraging, and we believe that these methods indicate an advance in clinical laboratory medicine and its can be a valuable tool for routine laboratory diagnosis of infectious diseases

B-066

Prevalence Epidemiologic Study of HCV Genotype in HIV-HCV Co-infected Patients

O. D. Romano, B. R. Rodrigues, M. A. Pereira, D. A. G. Zauli, F. L. O. Marinho, E. Mateo, A. C. S. Ferreira. *Hermes Pardini Institute, Vespasiano, Brazil*

Background: According to the World Health Organization, it is estimated that 34 million people are currently infected with human immunodeficiency virus (HIV) worldwide and 25-30% of HIV-positive individuals are co-infected with hepatitis C virus (HCV). In Brazil, HCV was reported to be present in 10.3% of HIV cases in 2010 and among these cases HCV genotype 1 was predominant, followed by genotypes 3, 2, 4 and 5. Individuals between 40-44 years old had the largest number of co-infection cases inthat year. HIV and HCV share the same infection pathway sand the use of injection drugs, blood transfusion and sexual transmission are the primary means of infection. Different HCV genotypes vary in infectivity and pathogenicity influencing, therefore, the rate of progression to cirrhosis and cancer, disorders that have an increased risk in HIV-HCV co-infection. Furthermore, HCV genotyping is necessary for the selection of patients who will respond better to treatment of HIV-HCV co-infection. Objective: To investigate the HCV genotype prevalent in HIV-HCV co-infected patients. Method: The study is a prevalence epidemiologic study of HIV-HCV co-infected patientsdata with HCV genotype determined in Hermes Pardini Institute (Vespasiano, Minas Gerais, Brazil) in 2013. The inclusion criteria was the detection of HIV-RNA by polymerase chain reaction. 3,308 patients, 1802 male and 1506 female, mean age 50.6 ± 14.5, were included in this research. Results: 25(0.75%) of the patients evaluated were HIV-HCV co-infected. Among these, 16 had HCV genotype 1 (64.0%), 7 had genotype 3 (28.0%), 1 had genotype 2 (4.0%) and 1 had genotype 4 (4.0%). The median age of co-infected patients was 47 ± 9.60 years old, 23 patients were male (92.0%) and 2 were female (8.0%). Conclusions: Among the HIV-HCV co-infected patients, genotype 1 was prevalent, followed by genotypes 3, 2 and 4. The HCV genotype 1 patients usually have more rapid progression to AIDS than other genotypes. Further investigations should be conducted with more patients to elucidate if there is any interaction between HCV genotype and HIV-HCV co-infection, since HCV genotyping predicts the effectiveness of the treatment and, consequently, it becomes a key factor to support therapeutic decisions.

B-068

MULTI-ARRAY Assay to Discriminate Recent from Long-Standing HIV Infection

M. Stengelin, D. Roy, M. Higgins, Z. Karuman, I. Maxwell, E. N. Glezer, J. N. Wohlstadter. *Meso Scale Diagnostics, LLC, Rockville, MD*

In order to accurately assess and compare different prevention strategies, the rate at which new HIV infections are acquired in a population needs to be measured accurately. A simple laboratory test that indicates whether an HIV infection was acquired in the recent past (generally 4-12 months) would be very useful to estimate HIV incidence.

We demonstrated feasibility of several assay formats to separate recent from longstanding HIV infection. Using MULTI-ARRAY technology, we measured antibodies against the HIV proteins gp41, gp120, gp160, p17, p24, p55, p66, and nef in a multiplexed format using a very small sample volume (25 µL of a 1,000-fold diluted serum or plasma sample). We used the well-characterized "HIV Incidence/Prevalence Performance Panel" from SeraCare (part # PRB601), which contains plasma samples from 15 HIV positive donors that have been characterized either as "incident" (recent infection) or "prevalent" (longstanding infection) based on consensus results from nine tests. Our MULTI-ARRAY serology format for antibodies against gp120 and gp160 showed ~10-fold separation between the median signals for incident and prevalent samples (and another ~10-fold separation from apparently healthy controls). All samples in each of the three groups were completely separated from the other two groups. We also developed avidity assay formats for antibodies against gp41, gp120, gp160, and p66 that could accurately separate samples from patients with incident versus prevalent HIV infection. The assays were developed in a 96-well high-throughput assay format for the MESO® SECTOR S 600 Imager and the MESO QuickPlex® SQ 120.

We demonstrated feasibility for transfer of the assay format to a point-of-care (POC) platform. The POC assay is fully automated and simultaneously measures concentrations of antibodies against eight HIV proteins. Time to result is 25 minutes, and CVs are approximately 13%. The magnitude of the antibody response against gp120 and against gp160 accurately separates patients with incident HIV infection from patients with prevalent HIV infection, equivalent to the plate-based results.

In conclusion, we demonstrated feasibility for development of high-throughput and point-of-care assays to discriminate recent from longstanding HIV infection.

Acknowledgement of Source:

The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: HIV-1 Nef Protein (Cat# 11478).

B-069

HIV p24 Immunoassay with the Sensitivity of PCR Methods

M. Stengelin, D. Roy, A. Aghvanyan, J. Kenten, G. B. Sigal, E. N. Glezer, J. N. Wohlstadter. *Meso Scale Diagnostics, LLC, Rockville, MD*

Patients who have recently been infected with HIV contribute disproportionately to the spread of the disease. Viral loads are high in the first few weeks after infection, and newly infected patients are unlikely to be aware that they are infected and can spread the disease to others. Therefore, early detection of acute HIV infection is of great importance for public health. PCR methods are the gold standard with respect to sensitivity; they can detect as few as 60 HIV RNA copies per mL of serum or plasma (30 virus particles per mL). However, PCR technology is complex and expensive, and therefore not suitable for all settings. Immunoassays are simpler and cheaper, but the detection limit of current, 4th generation p24 immunoassays is only about 10 pg/mL, or approximately 250 million capsid proteins per mL. On a per virus basis, these immunoassays are several thousand times less sensitive than PCR testing, despite the fact that there are about 2,000 p24 capsid proteins per virus.

A next-generation electrochemiluminescence assay format based on MSD's MULTI-ARRAY® technology was developed and its performance characterized. The detection limit for this novel p24 immunoassay was approximately 1 fg/mL, 10,000 fold more sensitive than current p24 immunoassays. A sensitivity of 1 fg/mL corresponds to less than 1 virus particle in our sample volume of 25 µL. The lower and upper limits of quantitation were 3 fg/mL and 38,000 fg/mL, respectively. Within-plate CV was 7%, and total CV 15%. Spike recovery and dilution linearity were between 80% and 120%. p24 was undetectable in the serum or plasma of 32 apparently healthy donors. A SeraCare p24 "Mixed Titer Panel" (12 samples) showed good correlation between our p24 assays and commercial p24 immunoassays. Two seroconversion panels were tested: SeraCare PRB948 (days 0 and 18, PCR negative; days 22 and 23,

PCR positive) and PRB962 (days 0 and 2, PCR negative; days 7, 9, 14, and 17, PCR positive). In both cases, the MSD® p24 assay result was negative for all PCR-negative samples and positive for all PCR-positive samples, and infection was detected well before conventional p24 immunoassays.

In conclusion, we developed a next-generation p24 immunoassay that is 10,000 times more sensitive than the current limits of p24 ELISAs and comparable in sensitivity to PCR assays. The assay does not require specialized equipment and can be run on the MESO® QuickPlex SQ 120, and all MESO SECTOR® Imagers.

B-071

Rapid Detection of Microbial Resistance to Lactam Antibiotics by LC-MS/MS

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Background:

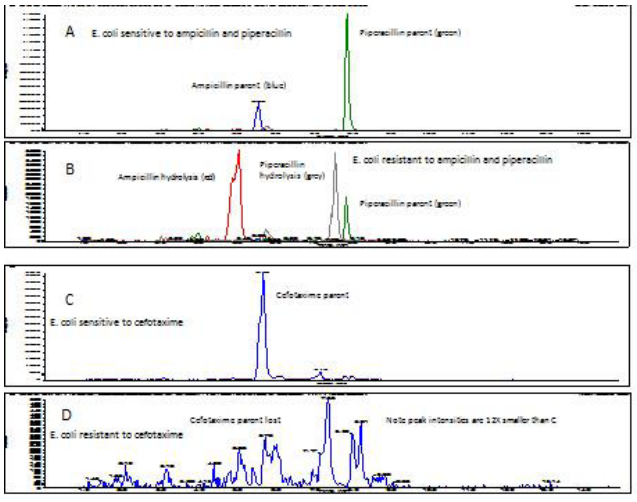
Bacterial sepsis and septic shock are major causes of mortality worldwide. In the US it is estimated that 250,000 patients a year develop life threatening infections with a mortality rate that varies from 28 to greater than 50% depending upon other underlying disease conditions and the severity of infection. MALDI-TOF mass spectrometry has revolutionized bacterial identification based on patterns of ribosomal protein expression. The determination of bacterial resistance to antibiotics by conventional turbidometric, spectrophotometric or disk diffusion methods which evaluate bacterial growth in the presence of antibiotics is still a relatively slow process which often requires 12-24 hours of incubation. This process often delays the administration of targeted antibiotics.

Methods:

Here we have adapted a rapid screening process for identification of bacterial resistance to antibiotics by utilizing LC-MS/MS to quantitate concentrations of parent drugs and also detect hydrolysis products which result from beta-lactamase activity. The susceptibility testing can be accomplished in time periods as short as 90 minutes which includes incubation of bacteria with antibiotics and LC-MS/MS analysis. The antibiotics can be multiplexed for incubation with bacteria to minimize analysis time.

Results:

We have evaluated 23 different strains of E. coli by this method including ATCC reference (3) as well as clinical isolates (20) and achieved complete concordance with traditional methods. To date the following antibiotics have been tested: penicillin, ampicillin, amoxicillin, cloxacillin, piperacillin/tazobactam, and cefotaxime. All incubations are conducted in the absence and presence of tazobactam which acts as a control. LC-MS/MS analysis was conducted on an AB SCIEX 3200 QTRAP® system utilizing positive electrospray ionization, with MRM detection. Chromatographic separation was performed using a C18 reverse phase column, with a linear methanol gradient. A sample chromatographic profile is displayed in Figure 1.



B-072

Preliminary validation of saliva samples as clinical matrix on the ReEBOV™ Antigen Rapid Test Kit for the point of care detection of Ebola Virus Disease

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Background: Ebola virus (EBOV) causes severe and often fatal viral hemorrhagic fever (Ebola Virus Disease; EVD). The 2014-2015 outbreak of EVD in West Africa is the deadliest of its kind, resulting in +9500 deaths and prompting an international emergency response. During the course of this outbreak, a need became evident for an easy-to-use, point-of-care rapid diagnostic test (RDT) that can be performed in any clinical facility or field laboratory to aid in the rapid triage of suspect EVD cases. To assist in this effort the Viral Hemorrhagic Fever Consortium (VHFC.org) led by Tulane University and Corgenix Inc. accelerated the development timeline of the ReEBOV™ Antigen Rapid Test Kit. This dipstick-format lateral flow immunoassay incorporates recombinant EBOV VP40 antigen and VP40-specific polyclonal antibodies to detect EBOV VP40 antigenemia in whole blood or plasma samples. We have also initiated validation studies for clinical testing of saliva samples in collaboration with Oasis Diagnostics Corporation (Vancouver, WA) and present those findings here.

Methods: In collaboration with the FDA, we developed an analytical validation protocol for the ReEBOV™ Antigen Rapid Test for whole blood and plasma samples. This protocol was used as a guideline for additional saliva sample validation studies, including Limit of Detection (LOD) using spiked recombinant antigen, mock sensitivity & specificity, interfering substances, cross-reactant reactivity, and prozone effect. Donor saliva (human) was collected using the Super•Sal™ Universal Saliva Collection Device (Oasis Diagnostics Corp.) In addition, the test was evaluated in comparison to qRT-PCR on in vivo saliva samples from EBOV Zaire (Makona)-challenged non-human primates.

Results: The LOD of the RDT is 4.8 ng/test for VP40 antigen spiked into saliva. Specificity was 100% (20/20; CI 83.6 - 100%) and mock sensitivity at LOD and above was 100% (40/40; CI 91.2 - 100%). Limited cross-reactant testing revealed no reactivity with several inactivated human viruses including Dengue virus, Junin virus, RSV, influenza, parainfluenza, mumps, and measles. No interference was observed for several drugs in common use. Aspirin appeared to cause a false-negative reaction; however, antigen-spiked samples from two donors under regular aspirin therapy (one low dose, one high dose) tested positive. For prozone effect, high levels of spiked antigen did not generate false-negative results. In non-human primates, the saliva of infected individuals tested positive 6 days after initial challenge. Conclusions: These preliminary validation studies have demonstrated the ReEBOV™ Antigen Rapid Test is capable of EBOV VP40 antigen detection in saliva samples. Available analytical validation presented here meets or exceeds comparable studies previously conducted with whole blood and plasma samples. Those studies contributed to the ReEBOV™ Antigen Rapid Test Kit being granted FDA Emergency Use Authorization and the WHO listing the test as eligible for procurement in February 2015. This product has not been authorized for the use of saliva samples in the diagnosis of EVD. However, these findings warrant further analytical and clinical validation efforts.

B-073

Fully automated digital immunoassay for p24 on the Simoa HD-1 Analyzer with the sensitivity of nucleic acid amplification for acute HIV infection

C. Cabrera, Y. Chen, L. Chang, D. H. Wilson. *Quanterix Corporation, Lexington, MA*

Background: Nucleic acid amplification techniques such as PCR have become the mainstay for ultimate sensitivity for detecting low levels of virus, including human immunodeficiency virus (HIV). As a sophisticated technology with relative expensive reagents and instrumentation, adoption of nucleic acid testing (NAT) can be inhibited in settings in which access to extreme sensitivity could be clinically advantageous for

early detection of HIV infection. We report a simple low cost digital immunoassay for the p24 capsid protein of HIV based single molecule array (Simoa) technology. The assay exhibited over three logs greater sensitivity than conventional immunoassays, and comparable sensitivity to NAT for early detection of HIV infection.

Method: Reagents were developed for a paramagnetic bead-based ELISA for use in the Simoa HD-1 Analyzer. Anti-p24 capture beads were prepared by covalent coupling of antibody to carboxy paramagnetic microbeads, detector antibody was biotinylated by standard methods, and an enzyme conjugate was prepared by covalent coupling of streptavidin and [[Unsupported Character - Symbol Font β]]-galactosidase. The HD-1 Analyzer first performs a 2-step sandwich immunoassay using 144 µL of serum or plasma sample, then transfers washed and labeled capture beads to a Simoa disc where the beads are singulated in 50-femtoliter microwells, sealed in the presence of substrate, and interrogated for presence of enzyme label. A single labeled p24 molecule provides sufficient fluorescent signal in 30 seconds to be counted by the HD-1 optical system. At low p24 concentration, the percentage of bead-containing wells in the array with a positive signal is proportional to the amount of p24 present in the sample. At higher p24 concentration, the total fluorescence signal is proportional to the amount of p24 in the sample. The concentration of p24 is then interpolated from a standard curve (range 0-30 pg/mL). Time to first result is 69 minutes. The assay was evaluated for sensitivity, specificity, precision, recovery, linearity, and correlation to a NAT method across 24 early HIV infection serum samples (prior to seroconversion).

Results: Limit of detection (2.5 SD) was 0.0025 pg/mL across 10 runs. This corresponds to ~60 RNA copies/mL equivalents by NAT. Limit of quantification (20% dose CV of diluted serum samples) was 0.0076 pg/mL across 11 runs and 58 determinations. Specificity was 95.1% across 139 normal serum and plasma samples. Recovery of p24 spiked into normal serum averaged 84.1%. Linearity per CLSI EP6-A guideline averaged 102.2%. Precision per EP5-A guideline included three serum-based panels and two p24 controls assayed in replicates of three at two separate times per day for five days using a single calibration curve. ANOVA gave CV's <10% for all levels. Method comparison to NAT yielded 100% concordance with a R of 0.961. Samples ranged from 40 to 10 million RNA copies/mL as reported by NAT, most of which were non-reactive by 4th generation HIV combo immunoassay.

Conclusion: The results show the digital Simoa p24 assay exhibited comparable sensitivity to NAT, as well as good general analytical properties. The assay represents a potential alternative to NAT for early detection of HIV infection.

B-074

Analytical validation of the ReEBOV™ Antigen Rapid Test Kit for the point of care detection of Ebola Virus Disease

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Background: Ebola virus (EBOV) causes severe and often fatal viral hemorrhagic fever (Ebola Virus Disease; EVD). The 2014-2015 outbreak of EVD in West Africa is the deadliest of its kind, resulting in +9500 deaths and prompting an international emergency response. During the course of this outbreak, FDA and WHO approved qRT-PCR as a molecular diagnostic to detect EVD clinically, however with this technique a result is not available for 24hr or more and requires significant laboratory infrastructure and electricity. A need became evident for an easy-to-use, point-of-care rapid diagnostic test (RDT) that can be performed in any clinical facility or field laboratory to aid in the rapid triage of suspect EVD cases. To assist in this effort the Viral Hemorrhagic Fever Consortium (VHFC.org) led by Tulane University and Corgenix Inc. accelerated the development timeline of the ReEBOV™ Antigen Rapid Test Kit. This dipstick-format lateral flow immunoassay incorporates recombinant EBOV VP40 antigen and VP40-specific polyclonal antibodies to detect EBOV VP40 antigenemia in whole blood (capillary and venous) or plasma samples.

Methods: In collaboration with the FDA, we developed an analytical validation protocol for the ReEBOV™ Antigen Rapid Test. Validation studies included, Limit of Detection (LOD) using EBOV Zaire virus and recombinant VP40 antigen, specificity, interfering substances, nearest-neighbor & cross-reactant reactivity, prozone effect, reproducibility, and stability studies.

Results: The LOD of the RDT is 3.00E+04 PFU/test for EBOV Zaire virus and 1.88E+01ng/test for recombinant VP40 antigen when spiked into whole blood. Specificity is 97.5% (39/40; CI 86.8 - 99.9%) for fingerstick (capillary) whole blood and 94.9% (131/138; CI 89.8 - 97.9%) against a US normal serum panel. Nearest-neighbor testing demonstrated that the RDT is reactive to all known African strains of EBOV (Zaire, Sudan, and Bundibugyo) that cause human infection; Ebola-Reston and Marburg virus were non-reactive. Cross-reactant testing revealed no reactivity with several human viral, bacterial and parasitic pathogens including Dengue virus, HIV, influenza, and malaria. No interference was observed for several drugs in common use including anti-febrile medication. However, high levels of hemoglobin and rheumatoid factor did interfere with signal development and are listed as limitations of the assay. No prozone effect is observed with high levels of spiked antigen or high titer clinical samples. Reproducibility testing demonstrated +95% agreement by multiple readers at the LOD and above using both spiked antigen and pooled clinical samples. On-going accelerated stability testing indicates a kit shelf-life of 12 months.

Conclusion: These validation studies have demonstrated the ReEBOV™ Antigen Rapid Test is a sensitive, specific, reproducible, and stable test for the detection of Ebola virus VP40 antigen in whole blood and plasma. Based in part on these findings, the ReEBOV™ Antigen Rapid Test Kit was granted FDA Emergency Use Authorization and the WHO listed the test as eligible for procurement in February 2015. The intended use is for the presumptive detection of Zaire EBOV in individuals with signs and symptoms of EBOV infection in conjunction with epidemiological risk factors. This Point of Care RDT represents a breakthrough in the detection of EVD for this and future Ebola virus outbreaks.

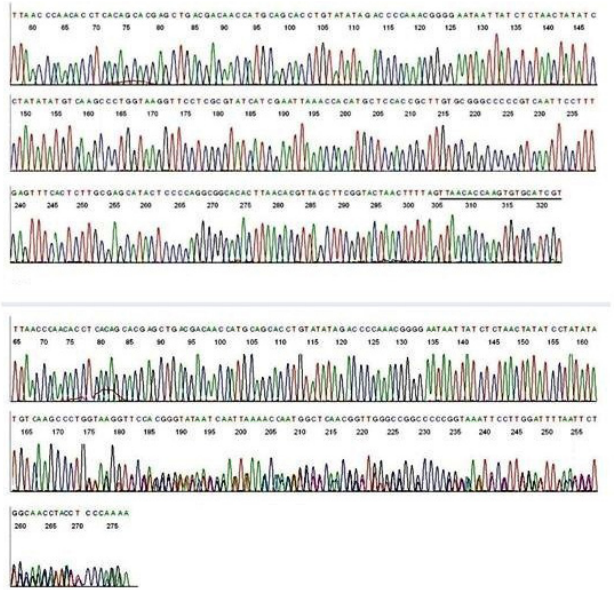
B-075

Direct DNA Sequencing Test for Lyme Disease Bacteria in Blood Samples

S. Lee. *Milford Molecular Diagnostics, Milford, CT*

Background Blood culture is not practical for the diagnosis of Lyme and related borrelioses. Various nucleic acid-based tests were developed for the detection of *Borrelia burgdorferi.* **Methods** A new pair of "genus-specific" primers, 5'-ACGATGCACACTTGGTGTTAA-3' (M1) and 5'-TCCGACTTATCACCGGCAGTC-3' (M2) was designed to amplify a 357-bp segment of the 16S ribosomal RNA gene (16S rDNA) of the *B. burgdorferi* sensu lato species and the correspondent 358-bp DNA segment of the relapsing fever borreliae, including *B. miyamotoi*. The crude DNA in NH_4OH extracts of the pellets spun down from patients' platelet-rich plasma or serum samples was the initial material for primary PCR amplification, followed by a same-nested PCR. The positive nested PCR products were used as the template for direct Sanger sequencing. **Results** Of 27 PCR-positive blood samples from clinically suspect "Lyme disease" patients, 19 contained *B. burgdorferi* sensu lato, 5 *B. miyamotoi*, 1 a mixture of *B. burgdorferi* sensu lato and *B. miyamotoi*, and 2 unnamed relapsing fever borreliae, including one with GenBank accession number KM052618. Of the 19 sequences of *B. burgdorferi* sensu lato isolates, 15 had a 100% ID match with a 16S rDNA signature sequence of *B. burgdorferi* senso lato, 3 had a single nucleotide mismatch against any closest sequence retrieved from the GenBank, and 1 showed more than one copy of 16S rDNA (Figure 1). **Conclusion** Direct DNA sequencing of the PCR amplicon of a highly conserved segment with hypervariable regions of the borrelial 16S rDNA may reveal greater strain diversity in the borreliae causing Lyme disease than previously estimated.

Figure 1 Upper: Signature sequence of *B. burgdorferi* sensu lato 16S rDNA. Lower: Many double base-calling peaks superimposed on each other downstream beyond the first 110 bases, indicative of more than one copy of 16S rDNA.



B-076

Performance of the VITROS ® Immunodiagnostic Products HIV Combo Assay*

C. A. Noeson, P. Contestable, A. Tweedie, L. Colt, C. Waasdorp. *Ortho Clinical Diagnostics, Rocheter, NY*

Objective: Assess the sensitivity, specificity and precision performance of the VITROS Immunodiagnostic Products HIV Combo Assay* on VITROS Systems with MicroWell capability. The assay is capable of simultaneously detecting both HIV antibodies (Ab) and p24 antigen (Ag) to enable earlier diagnosis of HIV infection.

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

All specificity and sensitivity testing was performed using one assay lot on a VITROS 3600 Immunodiagnostic System. Assay specificity was assessed using 2500 blood donor samples. Assay sensitivity was evaluated by running 8 commercially available seroconversion panels and 6 serially diluted patient samples. HIV-1 p24 antigen sensitivity was evaluated via serial dilution of AFSSAPS p24 standard. The assay was evaluated for total within lab precision over 20 days in accordance with CLSI EP05-A2 using one VITROS 3600 Immunodiagnostic System and one VITROS ECiQ Immunodiagnostic System.

Results: Donor specificity was determined to be 99.84% (95% CI: 99.59% to 99.96%) for blood donors. When used to test 8 commercially available seroconversion panels the HIV Combo assay was reactive at the same bleeds as a commercially available 4th generation assay. During antibody dilution testing the VITROS HIV Combo Assay* generated reactive results at least one dilution earlier than a commercially available 4th generation assay when evaluating high and low titer HIV-1 group M and low titer HIV-2 antibody. The VITROS HIV Combo Assay* generated reactive results within 1 dilution for HIV-1 group O antibody as compared to a commercially available 4th generation assay. The assay detects AFSSAPS p24 antigen at 15.2ng/mL. Within Lab precision of the assay ranged from 5.7 to 14.1% near the assay cut-off.

Conclusion: The VITROS HIV Combo Assay* enables earlier detection of HIV infection and provides comparable sensitivity and specificity performance to a commercially available 4th generation assay.

*Under Development

B-077

Prevalence and Antimicrobial susceptibility profile of microorganisms isolated from lower respiratory tract infections in hospitalized patients of Belo Horizonte - Minas Gerais/Brazil.

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Background: Lower respiratory tract infections (LRTI) have a great importance because of their high frequency and associated morbidity, and show high prevalence rates in Brazil and in the world, being the most prevalent hospital infections, mainly in intensive care units. In this study, the susceptibility profile information of local bacterial isolates (Belo Horizonte area) was evaluated aiming to contribute with a better understanding of the main bacterial types causing LRTIsin our region. This knowledge can contributeto an orientated antibiotic therapy and, possibly, a reduction of bacterial resistance. Patients/Methods:The epidemiologic study analyzed data obtained from electronic records collected between October 2011 to September 2012 of 584 patients suspected to have LRTI. The LTRI samples were collected by tracheal aspirate, mini-bronchoalveolar lavage and bronchoalveolar lavage. We identified microorganisms and evaluated their antibiotic sensitivity using the automated system MicroScan WalkAway® (Siemens, Erlangen-Germany). This technique involves the broth microdilution antimicrobial to determine the minimum inhibitory concentration (MIC). The identification of strains with possible antimicrobial resistance phenotypes was performed according to the recommendations of CLSI 2011. Results: From the 584 patients' data analyzed, 381 had positive cultures. The most prevalent microorganisms were Pseudomonas aeruginosa (25.9%) and Acinetobacter baumannii/haemolyticus (20.5%) followed by Staphylococcus aureus (11.3%, S. aureus MRSA corresponding to 45%), Stenotrophomonas maltophilia (5.7%), Klebsiella pneumoniae (4.4%) and Escherichia coli (4%).Regarding the susceptibility profile, we found a high level of resistance to the antimicrobials evaluated. 26% of Pseudomonas aeruginosa isolates were resistant to ciprofloxacin and levofloxacin, and 22% toaztreonam; and we also found resistance to the class of cephalosporins and carbapenems class, with 15% resistance to imipenem and 16% to meropenem. For the strains of Staphylococcus aureusmethicillin-sensitive, we found that 21% of the isolates were resistant to clindamycin, 24% to erythromycin and 60% to penicillin and ampicillin. In addition, 93% of Staphylococcus aureus (MRSA) showed resistance to clindamycin and erythromycin, 52% to gentamycin; 56% to levofloxacin; 33% to moxifloxacin and 4% to tetracycline. Escherichia coli strains showed increased resistance to ampicillin (43%) and to tetracycline (33%). Over 50% of the Acinetobacter baumannii strains showed resistance to most of the antimicrobial evaluated, highlighting amikacin with 66% of resistance, cephalosporins with about 80%, quinolones and carbapenems with approximately 90% of resistant strains. Conclusion: Given the importance of knowing the regional prevalence of microbiota and delineate an antimicrobial susceptibility profile, this study is of great importance to standardize the treatment regimen of LRTI in hospitals of the evaluated region.

B-079

Improved Detection of Histoplasma capulatum Antigen in Urine Specimens Utilizing Ultrafiltration and Commercially Available Enzyme Immunoassay Reagents

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Background: *Histoplasma* antigen detection in urine is useful for diagnosing and monitoring treatment of systemic histoplasmosis. Immuno-Myologic, Inc (Immy®, Norman, OK) has produced analyte specific reagents (ASRs) for a monoclonal based enzyme immunoassay for the qualitative /quantitative detection of this antigen. A literature search noted several studies where it was shown that the Immuno-Myologic assay demonstrated high specificity, but low sensitivity when compared to Mira Vista Diagnostics's proprietary Immunoassay, which some consider the industry's gold standard with regards to antigen detection. The aim of our study was to examine if ultrafiltration using Amicon Ultra-2 mL Centrifugal Filters would improve the detection and recovery of *Histoplasma* antigen.

Material and Methods: A total of 57 urine samples previously tested by Mira Vista Diagnostic were used for this study. 16 of the samples had antigen levels detected by Mira Vista at levels >0.4 ng/mL. The remaining 41 samples were below the established cutoff value and considered negative. The positive urine samples were tested neat with reagents from Immy to establish a baseline on a Dynex DSX 4-plate ELISA processing system. Following the analysis, these samples were concentrated

according to the manufacturer's instructions for Amicon Ultra-2 Centrifugal Filter Unit with Ultracel-3 membrane (EMD Millipore UFC200324). The concentrated samples were then tested, and samples that had the antigen concentrated to 0.4 ng/mL or greater were considered positive. The results were than compared to Mira Vista's results.

Results: Correlation between Immy's ASRs and Mira Vista for the Detection of *Histoplasma* antigen increased from 56.3% (9/16) positive agreement to 93.8% (15/16) positive agreement following ultrafiltration. Negative agreement remained the same, but did fall slightly from 100% (41/41) to 97.6% (40/41). Overall agreement with Mira Vista increased from 86.0% to 96.5% neat versus ultrafiltration, respectively. The urine sample was concentrated from a starting volume of 2.0 mL down to 150 µL, representing, mathematically, a 13-fold increase in concentration, but for unknown reasons, the amount that it was concentrated varied from sample to sample. The amount of antigen recovered after ultrafiltration ranged from 4-times to 29-times the original concentration.

Conclusion: The monoclonal enzyme immunoassay from Immy provides a unique opportunity for laboratories to test urine for *Histoplasma* antigen. The ability of laboratories to accurately test for

Histoplasma antigen can lead to a faster turnaround time and provide the physicians with useful information to make an initial diagnosis. However, the low sensitivity of the assay could mean that patients with low levels of *Histoplasma capsulatum* antigen may go undetected. Our findings suggest that the concentration of the urine sample by ultrafiltration would lead to a better detection rate of the antigen using Immy's ASRs. This method offers an accurate and sensitive method for qualitatively detecting *Histoplasma* antigen.

B-080

Bloodborne pathogen contamination in the era of laboratory automation and Ebola

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Background:

Guidance from the Centers for Disease Control (CDC) on laboratory testing for persons under investigation for Ebola virus disease has stated that routine laboratory testing can be safely performed using automated laboratory instruments by adhering to bloodborne pathogen practices. Many laboratories use total laboratory automation (TLA) systems to perform routine clinical testing wherein specimens are centrifuged, decapped, and transported uncapped on a conveyor belt to downstream test analyzers. TLA systems come with a range of safety features standardly available; furthermore, decontamination procedure recommendations from manufacturers has been variable during the 2014-2015 Ebola outbreak. To develop evidence-based protocols for handling high risk pathogens in the clinical laboratory, we sought to investigate the levels of contamination by common bloodborne pathogens Hepatitis B (HBV) and Hepatitis C viruses (HCV), of a TLA system occurring through routine clinical use as well as immediately after processing high-titer samples.

Methods:

In order to ensure that our clinical HBV and HCV assays, a laboratory-developed test (HBV) and Abbott RealTime HCV, were capable of nucleic acid detection from environmental specimens, we assessed the recovery of viral nucleic acid from swabs of non-porous surfaces (glass slides). We observed linear recovery of viral nucleic acid over a range of concentrations with a recovery of 75% and 46% for HBV and HCV, respectively. Following a risk assessment, environmental swabs were then performed at key locations along a representative TLA system, taken during routine clinical use. Contamination was further assessed immediately after running a small number of high-titer HCV specimens (mean 5.8 x 107 IU/mL); to distinguish this contamination from baseline contamination present prior to the experiment, clean glass slides were placed at key locations and swabbed for the presence of HCV, as above.

Results:

Of 79 baseline swabs performed on the TLA system, 10 were positive for HBV and 8 for HCV. Viral nucleic acid was consistently detected from swabs taken from the distal inside surface of the decapper discharge chute, with areas adjacent to the decapper instrument and the centrifuge rotor also positive for HBV or HCV nucleic acid. Of note, contamination was occasionally detected on exposed surfaces in areas without protective barriers between samples and personnel. After running known HCV-positive samples, at least one additional site of contamination was detected on an exposed area of the line after the decapper and next to a barcode reader.

Conclusions:

Together, these data indicate that a low level of viral contamination of automated clinical laboratory equipment occurs during clinical use and suggests a need for better risk-mitigation procedures when handling highly infectious agents such as Ebola virus. At our institution, we have coupled engineering controls and modified procedures with increased communication between laboratories and clinicians via on call Laboratory Medicine residents to increase safety of lab personnel while maximizing the test menu offered for patients with high risk pathogens.

B-082

Serum markers and microbial etiology of bacteremia

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Background: The microbial etiology of bacteremia determines the choice of adequate therapy for severe infections. The clinical manifestations of gram-negative and gram-positive bacterial infections are similar while biological markers may serve as a guide for the early diagnosis of the nature of a pathogen. The purpose of the study was to assess an association between the serum levels of lactate, procalcitonin (PCT) and C-Reactive Protein (CRP) and the microbial etiology of bacteremia.

Methods: We studied the role of these serum biomarkers in patients with gram-positive and gram-negative bacteremia. The PCT was analyzed by immunoassay in B.R.A.H.M.S.-KRYPTOR®, lactate and CRP was measured in DIMENSION EXL - SIEMENS® and blood culture was made in BACTEC-9240® blood culture system (Becton Dickinson). The program used for the data processing and statistical analysis was SPSS®.

Results: Our study included 77 patients, the median age was 64.5 years old (inter-quartile range (IQR): 53-71). Twenty-eight patients (36.4%) had bacteremia due to gram-negative bacteria and 49 (63.6%) due to gram-positive, with 38 isolations Staphylococcus spp were the most frequently isolated bacterium followed by Enterobacteria (11%), Escherichia coli (9.1%), Pseudomonas aeruginosa (9.1%) and Streptococcus pneumoniae (6.5%).

No statistically significant differences were found between gram-negative and gram-positive bacteremia according to the CRP levels ($p>0.05$). PCT levels were significant higher in the gram-negative bacteremia 6.23 ng/mL [IQR: 1.5-33.53] vs. 2.27 ng/mL [IQR: 0.48-27.6] in gram-positive, however lactate levels were significant higher in the gram-positive bacteremia 3.08 mmol/L [IQR: 1.65-4.85] vs. 1.09 mmol/L [IQR: 1.23-4.5] in gram-negative group. E. coli had the highest PCT value 27.06 ng/mL [IQR: 8.62-137.2] and S.pneumoniae had the highest lactate level 4.4 mmol/L [IQR: 1.5-5.7].

Conclusions: PCT and lactate showed diference between gram-negative and gram-positive bacteremia, it may be useful in differentiating the pathogenic bacteriemia and supposed the etiology before obtaining blood culture results.

B-083

Development and Validation of Robust Assay for Detecting Trypanosoma cruzi Parasites for Clinical Trial of Treating Asymptomatic Chronic Chagas Disease

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Background:

Chagas disease is caused by *Trypanosoma cruzi* (*T. cruzi*) infection. About 8 million people are estimated to be infected, primarily in Mexico, Central and South America. Left untreated, 40% of chronically infected patients will develop serious heart and digestive problems, with an associated 10 to 20% mortality for patients with severe cardiopathy. Current treatments with antiparasitic therapy such as benznidazole (BNZ) have limited efficacy in chronically infected patients and can cause serious side-effects. In 2011 Merck sponsored a Phase 2 proof-of-activity study of comparing posaconazole (POS), a triazole antifungal drug, to BNZ, in treating asymptomatic chronic Chagas disease. For primary trial objective, qualitative PCR results would be used to measure efficacy of treatments.

Methods:

PAXgene Blood DNA Tubes were selected over the traditional complicated method of collecting whole blood specimens from Chagas disease patients, which includes mixing 10 ml of blood with 10 ml of Guanidine Hydrochloride-EDTA followed by 10 minute boiling. PAXgene method allowed participating clinical sites in Latin

American countries to follow a simple protocol to collect and handle the specimens and to ship them at room temperature. However, PAXgene protocol required development of new methods to release parasitic DNA from blood and to extract DNA. Zymo Research's Quick-gDNA Blood MiniPrep kit was found to be able to efficiently release and extract *T. cruzi* DNA from PAXgene blood, without organic denaturants or Proteinase K digestion. After published PCR assays shown to have suboptimal sensitivity, we developed custom-designed TaqMan based qPCR assays to detect and quantify minicircle kinetoplast DNA (kDNA) from two strains of *T. cruzi*, representing the two lineages of *T. cruzi*. Linearized plasmid containing a sequence from *Arabidopsis thaliana* was spiked into the lysed blood before DNA extraction as internal control for evaluating DNA extraction efficiency and qPCR inhibition by potential blood heme contamination.

Results:

DNA extraction efficiency with PAXgene blood from healthy volunteers was high and consistent, with average efficiency of 93% with 7% CV. qPCR amplification efficiency for both *T. cruzi* strains, K98 representing Lineage I and CL-Brener (CLB) Lineage II, were > 90%. Assay precision for parasitic loads was good for both strains, with K98 ranging from 8% CV for 10 ppm (parasite per ml of blood) to 22% for 0.25 ppm, and CLB from 22% CV for 10 ppm to 27% for 1 ppm. The assay sensitivity, in term of limit of detection, which is the lowest ppm at least 95% of technical replicates can be detected, was 0.025 ppm for K98 and 0.05 ppm for CLB. Assay accuracy, in terms of measured ppm divided by known spiked-in ppm, were 103%, 99%, and 76% for 10, 1, and 0.25 ppm respectively for K98, and 123%, 97%, and 101% for 10, 1, 0.25 ppm respectively for CLB.

Conclusion:

A robust assay combining simple DNA extraction procedure with optimized TaqMan qPCR assay was validated for PAXgene blood specimens containing *T. cruzi*. The assay has high accuracy, sensitivity and precision and is being utilized to support the clinical trial for chronic Chagas disease treatment.

B-084

An Evaluation of Rapid Molecular Tuberculosis Identification with the Automated GeneXpert MTB/RIF System

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Background:

Tuberculosis (TB) is one of the major communicable diseases around the world and an estimated 9 million people were diagnosed based on the report from World Health Organization (WHO). With 12,338 new cases and 626 TB-related deaths in 2012, TB remains the highest incidence and mortality rate among all airborne contagious diseases in Taiwan. Conventional methods, sputum acid-fast bacilli (AFB) smear microscopy and culture of TB bacteria on liquid or solid media, can be inaccurate and time-consuming. The study aimed to determine the accuracy of the GeneXpert MTB/RIF system, a commercially available nucleic acid amplification technology, in diagnosing TB.

Methods:

A total of 469 sputum samples were collected in a medical center from November 2013 to November 2014. Lowenstein-Jensen (L-J) medium, Mycobacterium Growth Indicator Tube (MGIT), and GeneXpert MTB/RIF assay were performed to identify TB. Additionally, culture based conventional drug susceptibility test (DST) was administered to measure the efficacy of this molecular method.

Results:

The sensitivity and specificity of the GeneXpert MTB/RIF were 96.7% (59/61) and 98.0% (400/408) respectively. The negative predictive value (NPV) was 99.5% (400/402) and positive predictive value (PPV) was 88.1% (59/67). The MTB/RIF assay also detected 1 rifampicin resistant specimen and 3 susceptible specimens from the 469 cases, and the results were confirmed by drug susceptibility testing. Besides, the turnaround time for TB confirmation test decreased from 2-3 weeks to 2-3 hours in this microbiology laboratory.

Conclusion:

In conclusion, the GeneXpert MTB/RIF system exhibits high sensitivity and specificity for detecting tuberculosis and has the potential to apply to other human infectious pathogens. The study shows that the laboratory requires an efficient and accurate tool for identifying communicable disease. Timely confirmation of communicable diseases can lead to earlier treatment for the suspected individual, decrease the chance of transmission in the clinical settings, and provide more effective public health interventions.

B-085

Evaluating the Agreement between Architect-Abbott Chemiluminiscent Assay and Immunofluorescense Assay for Detection of Chagas Antibodies

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Background: Chagas disease, also known as American trypanosomiasis, is a potentially life-threatening illness caused by the protozoan parasite, Trypanosoma cruzi (T.cruzi). Chagas disease occurs mainly in Latin America. However, in the past decades it has been increasingly detected in the United States of America, Canada, many European and some Western Pacific countries. This is due mainly to population mobility between Latin America and the rest of the world. In our country the prevalence of Chagas disease is 4%. The diagnosis of Chagas disease can be made by observation of the parasite in a blood smear by microscopic examination. However, a blood smear works well only in the acute phase of infection when parasites are seen circulating in blood. Diagnosis of chronic Chagas disease is generally based on detection of anti-T.cruzi IgG antibodies testing with at least two different serologic tests. Blood screening is vital to prevent infection through transfusion and organ transplantation and, diagnosis of infection in pregnant women is essential. Objective: The purpose of this study was to estimate the agreement for determining anti-T. cruzi antibodies between the Architect Chemiluminiscent Assay (Candidate method) with a well-established Immunofluorescence Assay (Comparative method) in a group of individuals who were attended in our Laboratory in Argentina. Materials and methods: Serum samples were obtained from 41 healthy volunteers: 29 women and 12 men (aged 24-61 years) and from 22 individuals previously characterized as positive for anti-T.cruzi antibodies by other serologic tests (agglutination, ELISA), 10 women and 12 men (aged 23-65 years). Anti-T.cruzi antibodies were determined by CMIA Chagas ARCHITECT i1000SR Immunoassay Analyzer (Abbott Diagnostics) and Immunofluorescence Assay (Biocientífica S.A, Argentina).To estimate the agreement between the candidate method and the comparative method the CLSI guidelines EP12-A2, Vol. 28 N°3 was used. To compare the results obtained by the two methods the EP Evaluator® program was used, the percentage of agreement was calculated with a confidence interval of 95% and Kappa (κ) of Cohen coefficient was assessed on the scale of Landis and Koch. Results: The 41 healthy volunteers were negative by both methods, from the 22 individuals previously characterized as positive, 19 were positive by both methods and 3 were positive by CMIA Chagas and negative by Immunofluorescence Assay. Overall Agreement was 95.2% (86.9 to 98.4%); Positive Agreement 100.0%; Negative Agreement 93.2%; Cohen's Kappa 89.2% (77.2 to 101.1%). Conclusions: Our study, although small, suggested the use of a chemiluminescent immunoassay for anti-T.cruzi antibodies detection. The agreement between the results was assessed as 95.2%, Cohen's Kappa 89.2%, which indicates a high agreement. This way Immunofluorescence Assay could be replaced by the fully automated method as an initial test in the diagnostic algorithm of Chagas disease. The main advantages of CMIA Chagas ARCHITECT Immunoassay are: no experienced technician required, traceable and reliable alternative to more time-consuming procedures. In summary, the CMIA Chagas ARCHITECT Immunoassay characterized here is suitable for routine analysis in the clinical setting, is simple and rapid for anti-T.cruzi antibodies detection in serum or plasma.

B-086

Distribution of microorganisms detected in blood culture according to the presence or absence of neutropenia in cancer patients.

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Background:

Infection remains an important cause of morbidity and mortality in patients with cancer, and neutropenia has been recognized as a major risk factor for the development of infections in this population. The presence of neutropenia and other factors such as comorbid illnesses, presence the mucosa damage, catheters, and antibiotic use influence the spectrum of bacterial infection among cancer patients. Recognition of the epidemiological bacterial infection is important to guide appropriate empiric therapy.

Objective:

To evaluate the distribution of microorganisms detected in blood culture according to the presence or absence of neutropenia at the time of blood culture collected in cancer patients.

Methods:

A retrospective analysis of the records from the microbiology laboratory was performed to identify patients with blood cultures requested from July to September 2014. The study population was inpatients and outpatients from a private cancer hospital in São Paulo. Blood cultures were processed by the BacT/ALERT 3D® Microbial Detection System; positive bottles were smeared in common media plates and identified by Vitek MS® MALD-TOF, an automated microbial identification system that uses mass spectrometry technology to provide identification results in minutes. A positive blood culture was defined as the recovery of a microorganism(s) from one or more bottles from a blood culture set. The neutrophil count cell was checked at the same date that the blood culture was collected. Neutropenia was defined as an absolute neutrophil count (ANC) less than 500/mm^3.

Results:

Throughout the study period, a total of 5,499 blood cultures were obtained from 2,569 patients; 209 (8%) blood cultures were positive, 5 were polymicrobial. The microorganisms recovered from blood cultures included gram-positive (65%), gram-negative (34%), and yeast (1%). Coagulase-negative staphylococci (CoNS) was the most frequent isolated microorganism in neutropenic and non-neutropenic patients. *Escherichia coli*, *Klebsiella* spp. and Viridans streptococci were more common among neutropenic than non-neutropenic patients, although the difference were not statistically significant.

Conclusion:

Gram-positive were more common than gram-negative bacteria during the study period independent of the presence or absence of neutropenia. There was no significant differences in microorganism distribution between the groups of patients with neutropenia versus patients that did not had neutropenia at the time of blood culture drawn.

B-087

Analysis of cases of co-infection between HIV and syphilis in Brazil in 2014

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Background: Syphilis has been a public health problem known for centuries, while AIDS is a relatively new. Syphilis and AIDS co-infection is a complex clinical interaction and yet not well understood, because Syphilis significantly increases the risk of contracting HIV, and in turn, HIV can change Syphilis natural course.

In Brazil has been observed a substantial increase in the number of STD cases, such growth is mainly observed in cases of HIV and Syphilis, typically affecting similar patient groups, where coinfection can occur. Co-infection represents a potential risk for public health, as patients who practice unprotected sexual intercourse, with potential to increase the rate of new cases for both diseases.

This study aims to analyze the data of a large laboratory in Brazil, were patients were screened for both diseases.

Methods: Using the database of a large clinical laboratory we assessed 591626 patients results of HIV and 127946 patients results of syphilis during the year of 2014 from all the regions of Brazil.

All HIV test were performed by the methodologies of electrochemiluminescence immunoassay - ECLIA (HIV Combi PT COBAS®, Roche) and chemiluminescent microparticle immunoassay - CMIA (HIV Ag/Ab Combo Architect®, Abbott) both tests detect the HIV-1 antigen and total antibodies to HIV-1 and HIV-2, simultaneously, as a combo test. All syphilis test were performed by the methodology of chemiluminescent microparticle immunoassay - CMIA (Syphilis TP Architect®, Abbott) and confirmed by Fluorescent Treponemal Antibody-Absorption - FTA-ABS (FTA-ABS IFA Hemagen Diagnostics, Inc. VIRGO® Products Division).

Results: In all analyzed tests were verified 9790 HIV positive results and 40 534 positive cases of syphilis. Among the total number of patients we observed that 14 908 patients had a correlation of HIV, and syphilis tests. Of these, 1794 are syphilis positive, 140 are HIV positive and 125 have co-infection. In these 125 cases of co-infection 87.2% are males.

Conclusion: Analyzing at the correlation of positive cases, we realize that the incidence is low if compared to low number of co-infected patients, given the number of Syphilis cases diagnosed in Brazil.

This data shows that 94% of patients with positive syphilis are in risk patients. If we consider the total number of patients in this study, we can conclude that 34101 patients are at HIV infection risk, as syphilis infection, indicates that intercourse was done with no protection.

The absolute predominance of co-infected men (87.2%) may suggest that co-infected women are being under diagnosed because the clinical presentation of syphilis in men be more apparent (or easily detected) than in women.

B-088

A novel multiplexed qPCR assay for the detection of 10 bacterial and viral causes of meningitis.

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Background: Meningitis is an infection of the central nervous system that can have serious clinical outcomes. Viral meningitis caused by Enterovirus is very common, usually self-limiting and does not require treatment. Bacterial meningitis is associated with high rates of mortality and high frequency of severe sequelae, with patients requiring prompt treatment with antibiotics. Although the vast majority of cases of meningitis are viral, most patients with suspected meningitis are admitted to hospital and treated with antibiotics until a diagnosis of viral or bacterial meningitis has been made. The diagnosis of meningitis infection by culture is time consuming, lacks sensitivity, requires high-level technical expertise, and in the case of bacterial meningitis culture may return false-negative results especially after the initiation of antimicrobial therapy. Early identification of causative bacterial and viral pathogens may be important for prompt and proper treatment of meningitis and for prevention of life-threatening clinical outcomes. We have developed a panel of multiplexed qPCR assays to assist with the rapid detection of bacterial and viral agents commonly associated with meningitis.

Material and Methods: Multiplexed qPCR based assays were developed using MNAzymes for detection and differentiation of the most common causes of viral and bacterial meningitis in Cerebrospinal Fluid (CSF) samples. The assays consisted of four panels; (1) Bacterial panel 1 to detect N. meningitidis, S. pneumoniae and H. Influenzae, (2) Bacterial panel 2 to detect Group B streptococcus, E. coli and L. monocytogenes, (3) Viral DNA panel to detect Herpes Simplex Virus type 1 (HSV1), Herpes Simplex Virus type 2 (HSV2) and Varicella zoster (VZV), and (4) Enterovirus Viral RNA. Each of the panels additionally included an internal control to verify adequate sample processing of the target(s) and to monitor the presence of assay inhibitors. Performance characteristics tested for each assay panel included specificity, sensitivity, inclusitivity, linearity, impact of interfering substances and reproducibility. MNAzyme qPCR utilises multiple partial DNA enzymes or 'partzymes' that are inherently inactive, but when they combine in the presence of a target, they form active MNAzymes which cleave universal probes leading to signal generation indicating the presence of the target(s). MNAzyme qPCR possesses superior specificity and multiplex capacity compared to many other real-time chemistries.

Results: The four multiplex panels showed robust specificity, sensitivity, reproducibility with no inhibition of internal control signal. The R2 for all targets ranged from 0.97 to 0.99 and efficiencies ranged from 95% to 109%. No inter-panel cross-reactivity was observed with any components and no cross-reactivity was detected using a wide range of non-target organisms. The limit of detection (probit regression analysis, 95 % probability) ranged from 8 - 50 copies for each target organism. Signal detection was not affected by the presence of high levels of potentially intering substances found in CSF.

Conclusion: MNAzyme qPCR provides a flexible and unique approach to qPCR that is specific, sensitive, rapid and easily multiplexed. The assays developed are useful for rapid identification of important meningitis pathogens.

B-090

A first comparison study between two Fungus (1-3)-β-D-Glucan Assays for the diagnosis of invasive fungal diseases in China

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Background:

The study aims to evaluate the performance of the newly developed (1-3)-β-D Glucan Assay (G Test, Dynamiker Biotechnology (Tianjin) Co., Ltd.) with a side-to-side comparison with Fungitell® assay (Associates Cape Cod, Inc.) (ACC) in detecting invasive fungal diseases (IFD) using clinical serum specimen.

Methods:

21 clinically suspected IFD patient serum samples were collected from The First Affiliated Hospital of Guangzhou Medical University and a specific number was assigned to each sample. (1-3)-β-D Glucan detection was performed using ACC's Fungitell assay and Dynamiker's G Test. Fungitell kit is an FDA-cleared and CE-Marked diagnostic kit specialized for the detection of IFI. G test was recently approved by the CFDA authority and became available in the China market. Fungitell assay uses a positive cut-off value of 60-80pg/ml, as opposed to G Test which has a positive cut-off value of 70-95pg/ml. Moreover, the concentrations for the five standards of Fungitell assay are 31.25, 62.5, 125, 250 and 500pg/ml, while G Test's standard concentrations are 37.5, 75, 150, 300 and 600pg/ml respectively. The results were compared and analyzed using statistical tests.

Results:

Fungitell assay detected 11 positives (samples 1-9, 19 and 21), 9 negatives (samples 11-18, and 20) and 1 inconclusive result (sample 10). On the contrary, G test detected 11 positives (samples 1-10, and 21) and 10 negatives (samples 11-20). Positive results of G test that match with Fungitell assay are samples 1-9 and 21, while in samples 11-18 and 20 negative results were obtained from both kits.

Conclusion:

The test results demonstrated that the performance of Dynamiker's G Test is highly consistent with that of Fungitell assay with the total coincidence rate of over 90%.

Test results comparison between the two reagent kits.		
Reagent Kit	ACC Fungitell® assay	Dynamiker Fungus (1-3)-β-D-Glucan Assay
Cutoff value	60-80pg/mL	70-95pg/mL
Sample No.	Test Results	Test Results
1-9	+	+
10	+/-	+
11-18	-	-
19	+	-
20	-	-
21	+	+

B-091

Analyzing Amino Acids Profile in Influenza Virus Infection to Identify Potential Biomarkers for Clinical Applications

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Background: Influenza A viruses are major pathogens that cause respiratory infections and have considerable impact on human health yearly. It is not clear why some influenza viruses cause severe, even fatal diseases, while others cause relative mild respiratory infections. From our previous publication, we found that the CCR2+ monocyte plays a crucial role in the pathological outcome of highly pathogenic H1N1 influenza A virus infection. We also observed excessive accumulation of CCR2+ inflammatory monocytes in severe infection, which is correlated with type I interferons (IFNs) prolong production. Production of Type I IFNs in limiting viral replication is the first line of defense against virus infection. A lot of molecules are involved in type I interferon dependent immunity including toll like receptors (TLRs), myeloid differentiation factor-88 (MyD88) and interferon receptor α/β receptor (IFNAR). We have observed that amino acids concentrations in mice broncho alveolar fluid (BALF) are correlated with severity of influenza infection when using different pathogenic strains of viruses. The objective of this study is to investigate the amino acids profile in the IFNs related signaling pathway in innate immunity upon high pathogenic influenza infection by using knockout mouse models.

Methods: C57BL/6 (wild type) and three strains of knockout mice, CCR2 (-/-), MyD88 (-/-) and IFNAR (-/-), were used. Mice were anesthetized with Isoflurane and then infected by intranasal applications of 200 PFU of recombinant influenza A H1N1 virus A/PR8/34 (PR8). Infected mice were sacrificed on day 6 after infection. BALF was obtained by flushing airway three times with 0.5 mL sterile PBS and spun down to remove cellular debris. BALF amino acids were quantitated by ultra-performance liquid chromatography tandem mass spectrometry. Data were analyzed using a web-based server MetaboAnalyst.

Results: A significant difference was observed between these different strains of knockout mice when we analyzed the amino acids profile. The disease severity of knockout mice after infection was evaluated by the infiltration of leukocytes in pulmonary tissues. We observed that amino acids concentrations positively correlated with the disease severity. The uncontrolled viral replication leads to accumulation of CCR2+ cell in wild type mice, which help amplifying the inflammatory signal and lead to fatal outcomes of high pathogenicity virus infections. Interestingly, the BALF amino acids profile of PR8 infected CCR2 knockout mice is indistinguishable from naive mice. Obviously, CCR2+ monocytes are important cells involved in regulating the amino acids metabolism upon influenza virus infection. Principal Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA) enable a clear differentiation of different strain of knockout mice samples. The results suggested the regulation pathways for these molecules may not be the same.

Conclusion: The amino acids profile holds the potential to be diagnostic markers for highly pathogenic influenza virus infection. These findings provide insight into disease pathophysiology and can serve as the basis for developing disease biomarkers for influenza infection.

B-092

The Improvement in Blood Volume to Positively Impact on Bacteremia Diagnosis

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Background:

Blood culture volume is the most important variable in detecting bacteremia. However, the majority of hospitals in Taiwan do not meet the criteria for ideal volume during collection. The object of this study is to evaluate the relationship between blood volume and contamination rate, and furthermore time for positive by using the BD BACTEC FX blood culture system.

Methods:

Using BD BACTEC Blood Volume Monitoring system (Becton Dickinson, Sparks, MD) we know the average blood volume between 01/2013 and 06/2013 is 3.9ml; the ratio of 6ml or above blood among total collection is merely 10%, and the contamination rate is over 3%. All indexes are not fit in with acceptable criteria. From 07/2013 to 08/2013 we issued a questionnaire toward nursing department to understand their cognition in blood culture collection. To go on, we arrange a series of activities to correct their misunderstanding, including lecture and hands on training, mark blood drawing line on each blood culture bottle, video talking about standard operational procedure to perform blood culture collection was made for every new coming nurse, also to issue on intranet, randomly inspect their clinical practice, report of unusual indexes and collector's name to nursing department monthly. The nursing department also holds racing activity to promote low contamination concept. The positive rate and contamination rate along with blood volume were monitored monthly.

Results:

Start from June 2013 to December 2014, we initiated blood volume improvement plan to set 6ml blood per bottle as target. With implantation of educational program, continuous blood collection devices and automatic blood volume monitoring software, our 6ml achieve rate reach 100%, the blood volume increase from 3.9ml to 7.2ml; contamination rate decrease to 2.19%, average time to positive shorten for 3 hours.

Conclusion:

With our study we demonstrated that the education program is effective by significant volume increase, the collection of adequate blood volume is correlated with decreased contamination rates and faster recovery time of blood culture. By continuous educate phlebotomists on this concept, patients with bacteremia can be rapidly and correctly detected, benefiting both patients and hospitals.

B-093

Evaluation of maternal and congenital syphilis in six maternity hospitals in São Paulo, Brazil.

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Background:

Congenital syphilis (CS) is a major public health concern, even after the implementation of intervention protocols in several countries. Many of these women are not tested for syphilis or not treated adequately; about 40% of such pregnancies will end in miscarriage, stillbirth, or perinatal death, and surviving newborns are at risk for congenital malformations. The serological diagnosis of syphilis is based on nontreponemal tests (Veneral Disease Research Laboratory, VDRL, or Rapid Plasma Reagin RPR), which detect antibody to cardiolipin, and treponemal tests (such as fluorescent treponemal antibody-absorption, FTA-ABS, or hemagglutinin assay, TPHA), which detect antibodies specific to *T. pallidum*. The diagnosis of CS is based on laboratorial serology of women during the first and third trimester of pregnancy, and at the time of delivery. All infants potentially exposed to syphilis in utero based on the maternal serology should undergo serological testing for syphilis, regardless of maternal treatment. Asymptomatic infants whose mother received appropriate treatment should be followed monthly until their nontreponemal antibody disappears. If there is no documented successful maternal treatment during pregnancy or presence of signs or symptoms of CS, infants should complete clinical and laboratorial evaluation.

Objective:

To determine the frequency of positive syphilis tests among women and their infants at the time of delivery in six maternity hospitals in São Paulo, Brazil.

Methods:

A retrospective analysis of laboratorial records were performed to identify serological tests of syphilis among parturient and their infants attending in maternity hospitals in São Paulo, Brazil, during 2014. Parturient syphilis were tested by serological syphilis screening at the time of delivery; positive screening test was confirmed by RPR and, if RPR negative, by TPHA. In screened infants born to women with any previous positive serology for syphilis; positive screening test was confirmed by RPR plus FTA-ABS IgM (since maternal IgM does not cross placenta, its detection is indicative of active infection).

Results:

During the study, 1,628 mother/infant pairs with serological tests for syphilis were analyzed. A total of 67 (4.1%) women were diagnosed with syphilis infection. 60 (90%) of infants born to syphilis-infected women had positive screening test, and 18 (30%) were RPR positive. There were two cases (3%) of mother-to-child transmission of syphilis confirmed by positive FTA-ABS IgM.

Conclusion: Syphilis continues to affect large numbers of pregnant women. Our data are in agreement with an estimated 2% of seroprevalence of syphilis among pregnant women in São Paulo state, Brazil. To reduce cases of CS, it is important to ensure syphilis testing and treatment into routine antenatal programs. Besides, neonatal diagnosis with appropriate treatment and follow-up is mandatory, since the sensitivity of IgM serological tests have been reported been approximately 80% with a specificity of 90%.

B-094

Human Papillomavirus type 16 E7 attenuates AKT signaling and the downstream effector p70 S6 Kinase

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>**Background:** Papillomaviruses are small DNA tumor viruses that are the causative agents of squamous cell cancers in humans. Two of the viral proteins, E6 and E7, are required for the establishment and maintenance of human cancers. High risk E6 and E7 degrade p53 and Rb, respectively, which eliminates cell cycle checkpoints and drives cancer progression and viral replication. While the role of E6 and E7 in targeting p53 and Rb has been intensively studied, how E6 and E7 manipulate cellular signaling cascades to promote the viral life cycle and cancer development is poorly understood. The AKT signaling pathway is central to cell proliferation and overall cell survival, and is often upregulated in cancers.

Results: We hypothesized E6 and E7 could be activating the PI3K/AKT pathway in order to promote proliferation, anti-apoptosis, and overall survival of the cell. To our surprise, we observed the opposite. E7 proteins with high affinity Rb binding sites decreased phosphorylated AKT (pAKT), the active form of the kinase. This result was unexpected as pAKT levels are typically increased in transformed cells, including cervical cancer. Repression of pAKT by E7 was independent of the Rb and p130 degradation function of E7, but could be mapped to a novel domain in the C-terminus. E7 also decreased the activation of p70 S6K and 4EBP1, suggesting that E7 may induce a switch in the protein translation pattern of the cell. We hypothesize this phenotype promotes an environment primed to replicate and amplify viral DNA.

Conclusions: Decreasing pAKT, pp70 S6K, and p4EBP1 suggests that E7 is attenuating AKT activity in order to putatively alter protein translation in the cell. Previously, E7 has not been shown to alter AKT pathway in this manner, suggesting a novel function of E7. We hypothesize this decrease in AKT activity is to increase IRES dependent translation of particular cellular and viral proteins, which is the subject of further investigation. This translation shift could promote viral genome amplification and potentially an increased sensitivity to chemotherapeutics, due to the decreased AKT signaling. Our observed decrease in pAKT in primary keratinocytes is contrary to the observed increase in cervical cancers, but suggests the activation of AKT signaling could be acquired during the transition from initial infection to invasive carcinoma.

B-095

Development of Simultaneous Detection Lateral Flow Immunoassay Kit for GAS and ADV

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Background: Diagnosis of pharyngitis using a lateral flow immunoassay kit (LFIA) for group A streptococcus (GAS) antigen is widely used. About 15 - 30 % of cases in children of acute pharyngitis are caused by GAS. On the other hand, viruses (such as adenovirus (ADV)) are most common causes of acute pharyngitis.[1)] GAS pharyngitis and ADV pharyngitis is similar clinical features, but treatments is different. A simultaneous antigen detection of LFIA for GAS and ADV can assist the accurate diagnosis and reduce the physical burden of patients with minimizing specimen collection number of times. However, for the problem that the extraction process is different between GAS and ADV, these kit is not making a practical use as yet. We have developed a new simultaneous antigen detection of LFIA for GAS and ADV.

Method: A simultaneous antigen detection of LFIA for GAS and ADV kit consisted of four main components and one extraction buffer: extraction pad, conjugate pad based on colloidal gold, membrane pad, absorbent pad and a buffer including surfactants. The sensitivity and specificity of this kit were evaluated. As a positive control, inactivated GAS antigen and inactivated ADV antigen were diluted with running buffer mixing throat swab obtained from normal adults. As a negative control, three kind of viruses, bacteria two kinds are prepared. This kit is performed by the addition of 100µL of running buffers. This kit was read after 10min. We measured the intensity of the GAS detection line and ADV detection line, respectively by using densitometer.

Results: The analytical sensitivity of the kit when using inactivated GAS and ADV were in the range of 5 x 10^5org/mL to 2 x 10^7org/mL and in the range of 1ng/mL – 100ng/m, respectively. Intra CV is less than 15 %. No cross-reactivity in 5µg/mL influenza A virus, 5µg/mL influenza B virus, 5µg/mL RSV, 2 x 10^7 CFU/mL staphylococcus aureus, 2 x 10^7 CFU/mL streptococcus pneumoniae was observed.

Conclusions: Collectively, these data suggest our developed kit deserve inclusion in the diagnosis of pharyngitis. To our best knowledge, this is a first report of a simultaneous antigen detection of LFIA for GAS and ADV. This kit make high sensitivity, high specificity, simple to use and user friendly. We believe that this kit will be a useful tool for a rapid diagnostics of acute pharyngitis.

1) Bisno AL. Acute pharyngitis: etiology and diagnosis. Pediatrics 1996; 97:949-54.

B-096

Independent Verification of the Dynex M² Multiplexed Assay System Performance in the Democratic Republic of Congo Using Dried Blood Spots

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Background: A Dynex Technologies, Inc. Multiplier Flex System powered by M²® technology, a multiplexed chemiluminescent immunoassay platform, was utilized as the processing system for an MMRVT immunity assessment in support of the 2013 Democratic Republic of Congo Demographic Health Survey (DRC-DHS). In the course of that study 9978 dried blood spots (DBS) from children aged 12 - 59 months were processed. A multiplexed bead cytometry assay for Measles, Rubella and Tetanus was configured at the University of North Carolina and 754 result-blinded DBS independently processed in order to assess the validity of the M² results.

Method: For the M² system, 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. 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. For the bead cytometry system, antigen to Measles, Rubella and Tetanus were bound to carboxylated fluorescent microspheres using EDC - Sulfo-NHS attachment chemistry. 0.25" DBS punches were extracted into 1ml of PBS, 0.5% tween20, 5.0% dried milk and processed on each platform according to established ELISA processing protocols.

Results: Assay response for each system was calculated as negative-subtracted ratio to within-plate positive control. Pearson regression analysis provides R^2 values of 0.45, 0.61, and 0.55 for Measles, Rubella and Tetanus, respectively, with $p < 0.0001$. Cohen's kappa coefficient of 0.45, 0.93 and 0.69 for these assays indicate moderate, excellent and substantial agreement between platforms. Using the UNC data set as reference, M² sensitivity was shown to be 84.7, 98.5 and 88.7% with specificity of 65.4, 97.3 and 90.2%.

Conclusion: Independent assessment of equivalent DBS show a very good but assay-dependent levels of agreement between this flow cytometry and corollary Dynex M² panels. These data support the conclusion that the Dynex Multiplier Flex system powered by M² technology is a very robust system with excellent sensitivity and specificity. The use of this system in conjunction with DBS processing offers a cost-effective easy to use automated multiplexed immunoassay processing system in challenging environments

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B-097

Workflow characteristics of two random access molecular diagnostic instruments for Chlamydia trachomatis/Neisseria gonorrhoeae testing

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Background: Next Generation Molecular Diagnostic instruments are becoming increasingly flexible to provide workflow advantages to the clinical laboratory. One of these features, readily available on Clinical Chemistry systems but only recently recognized in molecular diagnostics, is Random Access, allowing laboratory technologists to load patient samples in a random and continuous manner without batch constraints, improving operational efficiencies and overall costs. These next generation Molecular Diagnostic instruments provide full automation to reduce labor and manual errors, consolidation of menu to run multiple assays at the same time and speed to release results quickly. Two instruments on the market that can deliver all these workflow benefits are the Panther System (Hologic) and the GeneXpert Infinity-80 System (Cepheid). The purpose of this study was to evaluate both instruments to determine which could deliver the broadest workflow advantages.

Methods: Workflow characteristics of each automated platform were determined based on testing 192 urine specimens with the respective manufacturer's *Chlamydia trachomatis/Neisseria gonorrhoeae* **(**CT/NG**)** assay. Utilizing Nexus, a company that specializes in time-motion studies, the hands-on and automation times were precisely measured by recording the start and end times of each step. Data was also collected for 96 urine samples within the 192 test run to compare the time required for lower and higher volume testing. The following parameters were compared for each platform: 1) hands-on time, 2) required return visits during processing, 3) time to first and final results and 4) instrument footprint.

Results: The GeneXpert Infinity-80 required more labor time for 96 tests (1 hour 52 minutes) and 192 tests (3 hours 44 minutes) than the Panther, which required 23 minutes of labor time at 96 tests and 33 minutes at 192 tests. Sample preparation and loading time were significantly longer on the Infinity-80 due to the manual pipetting of each sample into the GeneXpert cartridge prior to loading into the instrument. This required the operator to be vigilant and continuously working for the first 3 hrs and 44 minutes vs 27 minutes on the Panther System when running 192 tests. The Infinity-80 was quicker to first result (1hr 28 min) than the Panther system (3hrs 30 min) and could complete 96 samples faster (4 hours 3 minutes), while the Panther completed 192 samples faster (6 hours 54 minutes). The GeneXpert Infinity-80 has a larger footprint at 9 feet wide x 2.9 feet deep, whereas the Panther is 4 feet wide by 2.6 feet deep.

Conclusion: While both instruments offer random access sample loading, there are differences in the amount of labor, time and space required to operate each system. In clinical settings requiring relatively large sample throughput, such as demonstrated here with batch testing ≥ 96 samples, the Panther offers the advantage of reduced hands on time required for technicians to prepare samples. In contrast, the quicker time-to-result per sample may render the GeneXpert Infinity-80 more desirable in clinical labs having lower sample volumes.

B-098

Comparison of DiaSorin HBeAg and anti-HBe ELISA Methods: Generation of Combinable Data Using Automated Dynex DSX versus Manual Pipetting

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Background: As a goal for the laboratory, manual method assays are desired to transfer to an automated pipetting platform, thereby increasing efficiency and productivity. Therefore, Hepatitis Be Antigen (HBeAg) and Antibody to Hepatitis Be Antigen (anti-HBe) analyses by Enzyme Linked Immunosorbent Assay (ELISA) methods manufactured by DiaSorin S.p.A. were validated on the Dynex DSX platform, to be considered as a substitute for the existing qualitative manual ELISA platform using the BioTek ELx800 Gen5 Secure data analysis software.

Objective: The manufacturer states that if an automated instrument is used other than the instrument characterized in the package insert, the user is responsible for establishing their own assay performance characteristics. This study purpose was to make qualitative comparisons, evaluate precision, and to determine acceptance criteria for interchangeable use between automated Dynex DSX and manual HBeAg and anti-HBe assay performance.

Method: A correlation was performed with 90 serum samples for both analytes, evenly split between positive and negative results. Acceptability criterion within the EP Evaluator [Release 9 Semi-Quantitative Method Comparison] was defined as passing the Test for Symmetry and Cohen's Kappa >75%. Precision experiments were performed using 3 samples having low positive reactivity near the borderline cutoff. The precision samples were tested in 20 replicates for 3 days, for a total of 60 data points for each of the 3 samples, and assessed using the signal to cutoff ratio. Acceptance criteria for the precision experiments were: 1) Both assay processes generated precision data within ±15% agreement, or; 2) The automated DSX platform validation-derived precision values were less than manual pipetting historically-derived precision values. All experiments, whether processed on the DSX platform or pipetted manually, were read on the BioTek ELx800 using Gen5 Secure data analysis software.

Result: The correlation for HBeAg demonstrated 98.9% agreement with respect to qualitative interpretation, with a passing Test for Symmetry and Cohen's Kappa value of 97.8%. The intra-assay precision mean was 8.8%CV for the DSX and 7.6%CV for the manual method. The inter-assay precision mean was 11.5%CV for the DSX and 7.7%CV for the manual method. The historically-derived inter-assay precision was 16.3%CV. The correlation for anti-HBe demonstrated 94.4% agreement with respect to qualitative interpretation, with a passing Test for Symmetry and Cohen's

Kappa value of 89.4%. The intra-assay precision mean was 9.4%CV for the DSX and 10.3%CV for the manual method. The inter-assay precision mean was 9.9%CV for the DSX and 10.4%CV for the manual method. The historically-derived inter-assay precision was 9.7%CV.

Conclusion: The correlation and precision experiments for HBeAg and anti-HBe analyses by ELISA demonstrate acceptable combinability between samples that are processed by automated DSX platform or manual pipetting means.

B-099

Assessment of the Elecsys Anti-HCV II assay in three new seroconversion panels

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Background: Approximately 170 million people are chronically infected with the hepatitis C virus (HCV) and many more are undiagnosed. The availability of treatments that can cure most patients means that screening and diagnosis of HCV infection is a priority. The recently developed Elecsys Anti-HCV II assay has enhanced sensitivity (100%) and specificity (in routine samples 99.66/99.64% and in blood donor samples 99.84%) compared with comparator assays. In previous seroconversion panels, the Elecsys Anti-HCV II assay showed excellent seroconversion sensitivity. However, HCV is a rapidly evolving virus, with many different variations across genotypes, therefore, it is important that assays are continually assessed against the available seroconversion panels to ensure ongoing sensitivity and specificity.

Methods: Three seroconversion panels were recently released by SeraCare: PHV924 (collected over 88 days, genotype 2b), PHV925 (collected over 27 days, genotype1a) and PHV926 (collected over 14 days, genotype 3a). The Elecsys Anti-HCV II assay was performed with these seroconversion panels using the cobas e 601 platform. Results with competitor assays were recorded by SeraCare and are available with the seroconversion panel information pack.

Results: The signal/cutoff ratio for the Elecsys Anti-HCV II and competitor assays on each bleed date, for each seroconversion panel are presented in the figure. For panel PHV925 and PHV926 the Elecsys Anti-HCV II was positive strikingly earlier than the competitor assays. In panel PHV924 all assays were positive in the same bleed, however, there is a long period of time between donations 3 and 4 for this panel. The Elecsys Anti-HCV II assay also had a greater signal/cutoff ratio compared with the other Anti-HCV tests.

Conclusion: These data generated with the new seroconversion panels confirm that the Elecsys Anti-HCV II assay has excellent seroconversion sensitivity across HCV genotypes, allowing early detection of HCV infection.

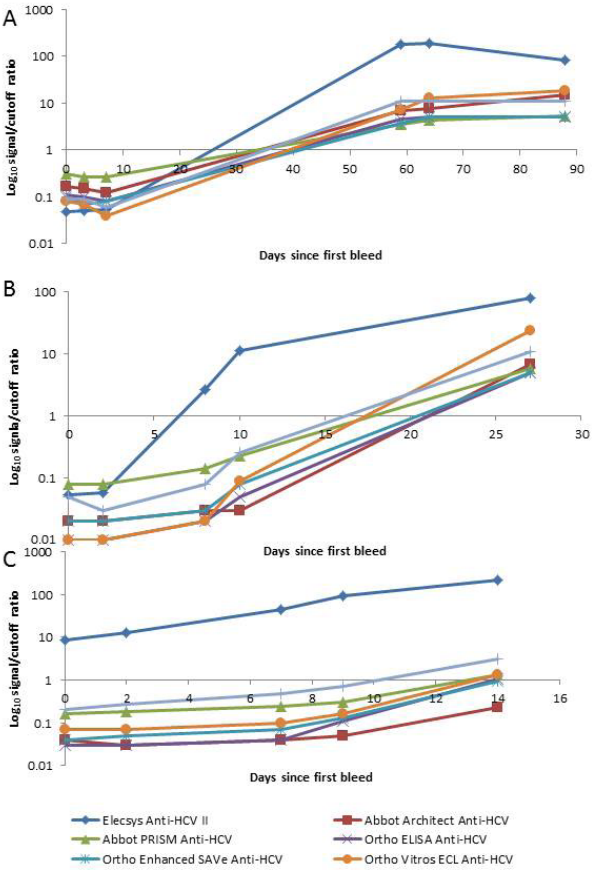

Figure. The log_{10} signal/cutoff ratio for each bleed day of the seroconversion panels A) PHV B)PHV925 and C)PHV926, for several anti-HCV assays. Values above 1 are positive results.

B-100

HIV-1 genotyping test by DNA sequencing: a new 'in-house' method

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The use of highly active antiretroviral therapy for the treatment of HIV has led to an increase in survival of HIV-infected individuals. However, due to a combination of extremely high level of virus production and a high mutation rate, HIV resistance to antiretroviral drugs is increasing, and can make such therapy ineffective over time. Therefore, close monitoring of HIV resistance is required and has been shown to improve antiretroviral treatment. Resistance testing may be performed by direct measurement of virus susceptibility to drugs, or through analysis of the sequence of genes encoding antiretroviral targets (genotyping). Genotypic resistance testing is currently recommended to aid the choice of new drug regimens following treatment failures. Aiming the development of an efficient and cost-effective assay the Research and Development (R&D) group and Genetics division of Hermes Pardini Institute (Belo Horizonte-MG/Brazil) established a new in-house assay. This genotyping method is based on DNA Sanger-based sequencing of the viral genome coding for protease (PR) and reverse transcriptase (RT), which has mutations associated with resistance. Three sets of highly conserved primers were designed to have a wide coverage, producing three overlapping fragments. Different HIV-1 subtypes and known drug resistance genotypes of the Hermes Pardini database were used for the validation. All sequencing reactions were performed using the BigDye Terminator kit (Applied Biosystem) on 3730 DNA Analyzer (Applied Biosystem). Sequences alignment against the HXB2 reference genome and analyses of the electropherograms were performed with the SeqScape software package version 2.5.The genotyping was performed by HIV Drug Resistance Database program (http://sierra2.stanford.edu/sierra/servlet/JSierra?action=sequenceInput) of Stanford University and the

results were compared with the genotyping previously determined by commercial TRUGENE kit (TRUGENE™ HIV-1 Genotyping kit/OpenGene DNA Sequencing System - Siemens). Results of the present in-house assay were 100% concordant with the results obtained with the TRUGENE, revealing the accuracy and specificity of our genotyping assay. Sequencing of three new molecular targets of HIV drug therapy - integrase, gp 41 and gp 120 - are being now added to these assay to improve the genotyping and consequently the clinical management of the patients.

B-101

In-house real-time quantitative PCR assay for the diagnosis and monitoring of hepatitis B virus and hepatitis C virus

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Acute and chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) lead to significant mortality and are a major public health problem worldwide. Over 350 million people are chronically infected with hepatitis B virus, and more than 170 million people areinfected with HCV worldwide.According to the World Health Organizationis estimated that 30% of infected patients develop liver cirrhosis and/or hepatocellular carcinoma (HCC).Diagnosis of hepatitis B and C infection isusually done by detection of anti-HBV/or anti-HCV antibodies against recombinant HBV or HCV proteins using enzyme immunoassay (EIA) and chemiluminescence immunoassay in the patient's serum. However, these markers have their own limitations hampering diagnostic accuracy. In order to overcome these problems, some studies reported in the last few years have demonstrated the development of molecular assays for detectionof nucleic acid-based markers related to hepatitis-causing viruses. The aim of the study was to develop anin house real time quantitative PCR (qPCR) assay to quantify the viral load of hepatitis B virus and hepatitis C virus in patient´s serum by using specific primers and TaqMan minor groove binder (MGB) fluorescent probe technology.Nucleic acids were extracted from serumusingQIAamp Virus Spin MiniElute Kit and Reverse transcriptionof viral RNA was performed withHigh Capacity cDNA Reverse Transcription Kit. The amplification reactions wereperformed in a 7500 Real Time PCR System, using the TaqMan detection system with predetermined concentrations of primers and probes,based on the amplification of a conserved region of the HBV and HCV genomes. For standardization and validation of the assay, an international panel of HBV/HCV and standard plasmids was used. A correlation coefficient of 0.98 and 0.96 for HBV and HCV, respectively, was obtained from Ct values and the concentration of DNA or RNA copies. The standard curve showed a linear relationship from 102 to 108 copies/mL of serum, with a coefficient of determination (r2) of 0.99and efficiencies of 90 - 100% for both HBV and HCV. In 116 clinical samples with a range of viral loads, the detection limit was of 103 copies/mL serum for both virus. The results suggest that the assay is suitable for viral quantification of low and high viral loads and that the amplification efficiency is stable over a range of input copy numbers. In conclusion, we developed a novel qPCR assay based on the TaqMan MGB system that is rapid, sensitive, and accurate. This assay, validated with both standards and clinical samples, provides an ideal system for routine diagnosis, monitoring of therapy and to confirm indeterminate serological results, especially in immunosuppressed patients

B-102

Prevalence and antimicrobial susceptibility profile of microorganisms isolated from blood cultures of hospitalized patients from Belo Horizonte/MG, Brazil.

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Background: The bloodstream infections (BSI) are the most relevant infections related to health care due to their high prevalence, high morbi-mortality rates and associated costs. They are among the most frequent and cause the most serious infectious complications of hospitalization and medical care. Regarding the severity and importance of the process, the correct diagnosis of bloodstream infections is extremely important and results in increased patient survival. Thus, the blood culture has significant predictive value when examinaning these infections. Methods: This study was carried out on samples of blood fo 1.394 patientes suspected to have bloodstream infection who underwent examination in laboratory from February 2011 to January 2012.The variables evaluated were the frequency of positivity in the samples and the sensitivity profile of microorganisms to antimicrobials agents. Three blood samples from each patient were collected by nursing staff and immediately inoculated into blood culture bottles for automated system BACTEC® (BD, New Jersey/USA). Regarding this system, the growth of microorganisms is detected based on the automatic detection of CO2 production in the culture medium bottles with fluorescent sensors. The identification and antibiogram were evaluated by the automated system MicroScan WalkAway® (Siemens, Erlangen-Germany). Results: 225 (16.1%) patients had positive blood culture for microorganisms. 553 microorganisms were isolated from the positive cases. Among patients with positive blood cultures, the mean age was 66.7 years(18-96 years old). The male/female ratio was 1.3 (55.8% male). The microorganisms most frequently isolated during this period were Staphylococcus aureus (n = 103; 18.6%), coagulase-negative Staphylococcus (n = 78; 14.1%), Escherichia coli (n = 60; 10.8%), Pseudomonas aeruginosa (n = 50; 9.1%), Acinetobacter baumannii (n = 39, 7.1%) and Klebsiella pneumoniae (n = 38, 6.9%). Fungi represented approximately 5.5% of the isolates. About the Staphylococcus aureus in blood cultures, 22% were Staphylococcus aureusmethicillin-resistant (MRSA). Regarding these, none was resistant to daptomycin, linezolid or vancomycin. However, among Enterococcus, 41% were resistant to vancomycin but none was resistant to daptomycin and 8% were not sensitive to linezolid. In relation tothe production of beta lactamase extended spectrum (ESBL), 20% of the strains of Escherichia coli (12/60), 38% of Klebsiella spp (16/42) and 39% of Proteus spp (12/31) produced this enzyme. Conclusion: The BSI prevalence data and its etiology should be the more regionalized and updated as possible, so that prevention actions can be more effective and concrete. This study contributed to the knowledge of the microbiological panorama of a large hospital in Belo Horizonte/Brazil, providing very important information for rational use of antimicrobial strategies and reduction of bacterial resistance.

B-103

Development and Evaluation of a Serological Chikungunya Antibody Detection Assay

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Background: Chikungunya (also named breakbone fever) is a highly emerging disease in many tropical settings with great socioeconomic impact. Causative agent for this disease is a single-stranded, enveloped RNA-Virus that belongs to the genera Alphavirus of the togavirus family (Togaviridae). In general the viruses are not transmitted from human to human but transmissions from infected pregnant women to unborn children have been proved. The symptoms of Chikungunya include fever which can reach 39°C (102,2°F) a petechial or maculopapular rash usually involving the limbs and trunk, and arthralgia or arthritis affecting multiple joints which can be debilitating. The symptoms can also include headache, conjunctival injection and slight photophobia. The fever typically lasts for two days and abruptly comes down. However other symptoms, namely joint pain, intense headache, insomnia and an extreme degree of prostration last for a variable period, usually for about five to seven days. But patients have complained joint pain for much longer time period depending on age of the patient. It has been observed that the severity of the disease as well as its duration is less in younger patients and pregnant women. Heavy damages to somebody's health or death are rare. Alphaviruses rarely appear in Europe but can be noticed as import or travel associated infection.

Methods: The aim of this work was to develop an serological assay to detect IgG and IgM antibodies against Chikungunya and to evaluate in endemic outbreak settings.

Results: An IgG-capture and IgM-capture ELISA was developed. Both take advantage of native antigens produced with a proprietary technique which was exclusively developed for this serological antibody detection assay. In house measurements as well as external evaluations in many endemic regions of the world conducted by well know tropical institutes revealed excellent clinical sensitivity and specificity as well as high positive and negative predictive values (all above 95%). Data from the current outbreak in the Caribbean will be discussed.

Conclusion: Therefore the newly developed ELISA seems to be a superior tool to diagnose past and acute Chikungunya infection in common and outbreak settings all over the world. It will assist diagnosis of travel returners with unknown fever as well as military in endemic operation area.

To further improve Chikungunya diagnostic a Lineblot is currently under development as tool for conformation of ELISA results as well as for small labs with limited lab equipment.

B-104

Multiplex sensitive type-specific detection of 14 high-risk HPV strains in a single closed-tube reaction

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Human papillomavirus (HPV) is one of the most common sexually transmitted infections (STI). Some, but not all, types of HPV can cause cervical cancer (high-risk HPV types or HR-HPV). Since early stage cervical carcinomas are nearly 100% curable, early detection is very important. Accurate molecular diagnosis is needed to inform patient management and follow-up treatment. We have developed a unique technology (Multiplex Probe Amplification or MPA) for sensitive type-specific detection of 14 HR-HPV types in a single closed tube reaction. In this study, we analyse the analytical and clinical performance of this novel HPV molecular diagnostics test.

Validation of the MPA HR-HPV assay was initially conducted using plasmids bearing type specific HPV sequence. Data showed that 100% of HPV plasmids were detected and genotyped correctly by MPA real-time PCR assays. Serial dilution of cell line DNA was also performed to evaluate the MPA real-time PCR sensitivity which showed that the assay can detect at a concentration of 10 copies per reaction. A pilot clinical evaluation of the MPA HR-HPV assay was conducted by investigation of HPV genotypes in 30 clinical samples. A comparison of MPA results with commercially available Luminex based genotyping assay showed 76.6% full concordance. Current work is looking at clinical evaluation of MPA HR-HPV assay on a set of 500 clinical samples from a screening population and its comparison with other commercially available clinically validated HPV assays.

In conclusion this study shows that the MPA HR-HPV assay is efficient in combining screening and genotyping of HPV-DNA. The current commercially available probe-based methods are limited to detection of only one target sequence per fluorescence channel. MPA technology overcomes this limitation, allowing 14 targets to be detected and quantified in a single closed-tube reaction. We believe that the MPA HR-HPV assay may offer a simpler and more cost effective means to identify women at risk and optimise treatment strategies.

B-105

Incidence rates of compulsory notification of reportable diseases in three different states of Brazil in 2013 and 2014 according to laboratory tests results

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Backgroud:

In Brazil, reportable diseases are responsibility of the Board of Health Surveillance of the Brazilian Federal Ministry of Health. The occurrence of new cases of a disease (transmitted or not) or injury (unusual or not), that can be prevented and controlled by health services, indicates that the population is at risk and may pose threats to health and need to be detected and controlled still in its early stages. The compulsory notification is the notification of the occurrence of individual cases, clusters of cases or outbreaks, suspected or confirmed, which shall be communicated to health authorities by health professionals or any citizen, aimed at the adoption of the measures relevant control. We present here a summary of the notifications for five main diseases in three Brazilian federation states during the years of 2013 and 2014.

Methods:

According to the federal standards for notification of reportable diseases data from three states were collected in this study: São Paulo, Paraná and Goias. The laboratory tests used for reporting the main diseases were: non treponemal antibodies tests and treponemal antibodies tests (syphilis), Western blot test (HIV), anti-HBc IgM and HbsAg (hepatitis B), Anti-HCV (hepatitis C) and Dengue IgM antibodies tests (dengue fever). Each state had its own data and all information from the years 2013 and 2014 were unified into a common file.

Results:

São Paulo had an average over ten thousand notification per year whereas Paraná counted three thousand and Goiás had a close rate of almost three thousand notification per year. The five main reported disease considering the 3 states were, Syphilis 14588 cases, HIV 4619, Hepatitis C 2727, Hepatitis B 2148, Dengue fever 1333.The number of tests processed for these five different diseases during this two years period were: Syphilis - 1,289,076; HIV - 10,994 Western blots, realized for 1,238,128 samples; Hepatitis C - 1,339,793; Hepatitis B - 1,324,068; and Dengue fever - 15,384.

Conclusion:

Syphilis was the most reported disease in the three states, with the highest positivity rate, followed by HIV, Hepatitis C, Hepatitis B and Dengue fever. The physician that receives the tests results ad integrate them with the clinical data is the most reliable source to notificate the reportable diseases. But large labs as DASA, that have access to millions of samples, can provide very useful information to the sanitary and surveillance authorities.

B-106

Blood culture candida isolates in tertiary public hospitals in southeast brazilian region

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Background: Candida is an opportunistic pathogen that affects high-risk patients who are either immunocompromised or critically ill and an increasing cause of bloodstream infection (BSI). Candida spp is currently between the forth and the sixth most common nosocomial bloodstream isolate in international studies and is associated with almost 80% of all nosocomial fungal infections. Candida albicans is the main cause of candidemia, but other species with more reduced susceptibility to antifungal agents has emerged as common pathogens. Importantly, identification of Candida isolates helps in selection of effective antifungal therapy.

Objective: The aim of this four-month observational retrospective tudy was to evaluate the distribution of Candida species of candidemia from eleven tertiary public hospitals in Brazil.

Methods: from October 2014 till January 2015 all cultures originated from blood specimens were processed in accordance with NCCLS - National Committee for Clinical Laboratory Standards - and incubated in Bactec (Becton Dickinson Inc.). Positive samples were submitted to identification including Gram stain and sowing in chromogenic medium (chromIDTM Candida, bioMérieux) for fungal isolation and identification of Candida albicans and other medically relevant candida species. The statistical analysis was performed with SPSS v18.0 software (IBM).

Results: During the study period were analyzed 2,710 samples and blood cultures positive for fungi was observed in 5.5% (149/2710) of the samples. The average time for positivity was 27 hours and the majority of patients were concentrated in two age groups ($p<0,001$): infants to 1 year (37.6 %) and above 60 years (24.2 %). Candida non-albicans was the more frequent isolates (59.1%) without significant distribution among month ($x2= 2,132$; $p=0.547$).

Conclusion: our study confirmed data from previous studies that demonstrated high prevalence of BSI by Candida spp in Brazil caused by species other than C. albicans, and shows that Candida non-albicans was the main isolated agent in nosocomial fungal infections. Use of chromogenic medium allowed a rapid identification that is important to effectiveness of fungal therapy.

B-109

Seroprevalence of HIV I and II in blood samples processed in a clinical pathology reference laboratory in Brazil between 2008 and 2010.

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Background:The epidemic of infection with human immunodeficiency virus (HIV) is a global, dynamic and unstable phenomenon, and thoroughly discussed by the scientific community and society in general. This disease is considered the first great pandemic of the second half of the twentieth century and was first described in 1981. In Brazil, the first cases of AIDS were reported in 1987. The transmission, as far as we know, is through contact of mucous with body fluids, such as blood or blood products, semen and breast milk.

Objective: We aimed to statistically analyze the serological results of HIV I and II processed at Hermes Pardini Institute (IHP) in the 2008 - 2010 period and describe the epidemiological characteristics of HIV-positive patients.

Method:This was a retrospective study, carried out through consultation of laboratory test results stored in IHP web LIS. All results of an HIV I and II obtained and released

from January 2008 to December 2010 were compiled. Epidemiological data such as gender, age and region of the country of HIV-positive patients were statistically analyzed and compared with national data from the Ministry of Health (MH) - Epidemic Update 2011.

Results: We evaluated 816,922 results from all over the country between 2008 and 2010. There was a predominance of patients from the Southeast region (59.5%), home of the laboratory, followed by the Northeast (21.1%), North (8.2%), South (7.8%) and Midwest (3.4%). The annual rates of seropositivity for anti-HIV I and II were 1.52% (2008), 2.32% (2009) and 1.11% (2010). Among HIV-positive cases, 8.2% were children (under 20 years), 88% adults (between 20 and 60 years) and 3.7% elderly (over 60 years). There was a male predominance in adults and the elderly patients. In the pediatric population, the positivity was higher in women. In the Southeast and Midwest, serology was mostly positive in men while in the north, northeast and south, the positivity was higheramong women. Comparing the results from IHP with the data from the MH, there was epidemiological similarity concerning gender and age of seropositives.

Conclusions: The findings of this study allow us to infer that the epidemiological profile of seropositive for anti-HIV I and II antibodies assisted by IHP reflect the Ministry of Health data regarding the sex and age of the Brazilian population. The differences found in relation to regionalities may be caused by the IHP assistance coverage throughout the national territory.

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Distribution of nearly 100,000 positive urine culture performed in Brazilian private hospitals

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Background:

Urinary tract infections (UTIs) represent the second most often observed infections in outpatients, and the most frequent infection among inpatients. UTIs are more frequent in female than male patients, particularly between 20 to 60 years-old. Complicated urinary tract infection occurs in women or men of all ages with functional or structural abnormalities of the urinary tract. The UTI diagnosis is based on medical history, urinalysis and the bacteriological urine culture with identification of the causative agent. The most common bacterial cause of uncomplicated community-acquired UTI is *Escherichia coli*. In nosocomial UTIs, the most frequent pathogens are *Proteus spp*, *Klebsiella spp*, *Enterobacter spp*, *Serratia spp, Pseudomonas spp*, *Enterococcus spp*, and *Candida spp*.

Objective:

To evaluate the culture of urine samples from patients attending 10 private hospitals in São Paulo, Brazil, and analyzed the microorganisms distribution according to sex and age.

Methods:

The study included samples from patients attending ten private hospitals of São Paulo, the largest city of Brazil. Retrospective analysis of all urine samples collected in 2013 and 2014 from inpatient and outpatients for bacteriological cultures were performed. All urine samples were smeared in the hospital where they were collected and, afterwards, sent to analysis in a central microbiology laboratory to be processed. Positive urine culture was defined as culture with >100,000 CFU /ml.

Results:

During the timeline of the study, 18,698 (19%) of a total of 97,962 urine cultures were positive; the proportion of positive culture among women was higher (23%) than among men (12%) ($p<0,001$). The percentage of positivity was 7% among < 1 year-old patients, 10% among 1 to 19 year-old ones, 23% among patients 20 to 60 year-old, and 26% in over 60 years-old. The female-to-male rate was higher in 20 to 60 years-old patients (6,5), than 1 to 19 year-old (4.5), < 1 year old (1.5) and over 60 years-old patients (2.0).

Gram-negative bacteria represented 85% of total of isolated microorganisms and *E. coli* was the most prevalent isolated microorganism. The most prevalent microorganism among female were *E. coli* (62%), *K. pneumoniae* (9%), *P. mirabiis* and *S. saprophyticus* (5% each). Among male, the most prevalent microorganism were *E. coli* (35%), *K. pneumonia* (14%), *P. aeruginosa* (13%), and *P. mirabilis* (9%). *C. albicans* was responsible for 2% of total UTIs, with no difference between sex.

Conclusion:

Although females are more prone to infection than males, the female-to-male rates are lowest in the extremes of age. The percentages of positive urine culture increase by age, from 7% among young than1-year-old to 26% among older than 60 year-olds. The distribution of the microorganisms varied according age and sex. The distribution of strains are in agreement with recent studies where Gram-negative microorganism being represented by the *Escherichia coli*.

B-111

Epidemiology of Respiratory Virus among Patients Attending Private Hospitals in São Paulo, Brazil.

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Background:

The respiratory viruses are the main cause of acute respiratory infection and are responsible for high levels of morbidity particularly in children, elderly people, and persons with comorbidities and immunosuppressive conditions. Most respiratory viruses present with similar symptoms, making a diagnosis difficult without laboratory testing. Although rapid antigen testing can offer quick results, the specificity and sensitivity of this testing vary greatly. Molecular biology techniques are capable to detect a panel of respiratory virus with a higher sensitivity.

Objective:

The aim of this study is to evaluate the distribution of respiratory viruses isolated during 2014 in patients from São Paulo hospitals and the seasonality pattern.

Methods:

Records from respiratory virus panel test results performed in 2014 were evaluated. Respiratory viruses were investigated from nasopharyngeal swabs of patients attending private hospitals in São Paulo city, Brazil. The exams were indicated based on attending physician decision in order to establish the etiology of respiratory infections. The test utilized was RT-PCR Microarray CLART® Pneumovir detecting 18 respiratory virus: Influenza A (H3N2, H1N1, H1N1pdm 2009, Influenza B and Influenza C, Parainfluenza (PIV) 1, 2, 3 and 4, Syncycial Respiratory Virus (SRV) A and B, Rhinovirus (HRv), Adenovirus (Ad), Bocavirus (HBoV), Metapneumovirus (HMPV), Coronavirus (CoV), and Enterovirus (Echovirus, Cosackievirus A and B).

Results:

Respiratory virus was detected in 1,689 (66%) of 2,569 nasopharyngeal swabs samples analyzed. A total of 528 samples identified more than one respiratory virus from the same patient specimen: 436 detected two, 79 detected three, 12 detected four, and one sample detected five respiratory viruses. A total of 2,323 respiratory viruses were identified. The most frequent respiratory virus detected were SRV (A and B, 27%), HRv (24%), HBoV (12%) and HMPV (9%). Results for both gender and all age groups were similar for all respiratory virus detected. The positivity of the total samples was higher in the fall (35%) and lower in the summer (17%) period, a difference not statistically significant. All respiratory viruses were also detected more frequently in the fall, with a difference not statistically significant, compared to other seasons.

Conclusion:

During the study period, the majority of patients submitted to laboratory investigation for respiratory infection, in private hospitals, had at least one respiratory virus detected in nasopharyngeal swabs. Non-influenza respiratory viruses are more important and the present study highlight the contribution of molecular methods used to detect respiratory viruses in the epidemiological knowledge and management of patients with respiratory disease.

B-113

Correlation between Antifungal Treatment and Galactomannan Antigen in Adult Hematologic Patients at Risk for Invasive Aspergillosis

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Background:

To analyse the correlation between antifungal treatment and galactomannan antigen in adult hematologic patients at risk for invasive aspergillosis (IA) together with the results of serial serum Aspergillus galactomannan (GM) antigen testing.

Methods:

In a retrospective study for patients at high risk of aspergillus pulmonary infection, serum GM testing was used to detect GM concentration 2-3 times/week during the periods of high risk for IA. High-resolution CT was performed in case of abnormal chest X-ray and/or persistent fever after 5 days of antibiotic treatment. IA was

classified as either "proven" or "probable" in accordance with the definitions stated by EORTC-MS.

Results:

A total of 82 hematological patients were diagnosed of "proven" (n=1),and "probable" IA(n=28) , and "possible" (n=23) and "No" IFI(n=30) .The sensitivity of the Dynamiker's GM test was 84.6%, and the specificity 81.3%. The false positive rate was 18.8%, the false negative rate 15.4% and the diagnosis rate 82.8%. This group of 82 patients received prophylaxis fluconazole treatment at a median of days 19.7 (range 9- 26).

Conclusion:

Dynamiker's serum GM test could be taken 2-3 times/week in adult hematologic patients at risk for IA. The GM value was correlated to the amount and the fungal load in patients. The GM test is also earlier than the conventional CT or chest X-ray scan.

B-114

Cytomegalovirus and Neonatal Screening: A protocol adaptation of ELISA kit for the detection of IgM antibodies in total blood collected on filter paper.

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Congenital CMV infection, which occurs in 0.2 to 1% of live births worldwide, may result from transplacental acquisition of either a primary or recurrent maternal infection. Clinically apparent disease in the neonate is much more likely to occur after a primary maternal exposure, particularly in the first half of pregnancy. In some higher socioeconomic groups in the US, 50% of young women lack antibody to CMV, making them susceptible to primary infection.

This study is aimed to evaluate the use of a commercial kit with the ELISA methodology for the detection of IgM antibodies for cytomegalovirus (CMV), in blood samples from newborns, collected on filter paper. With this objective, we performed the adaptation of the original protocol of kit ETI-CYTOK-M reverse PLUS Diasorin, indicated for the qualitative determination in human serum or plasma of IgM antibodies for CMV. The modification consisted of extracting 2 spots of filter paper containing blood of a newborn, directly to each microplate and making the elution of the total blood as a previous step before the original procedures described on the test datasheet.

The validation process used 40 samples, 11 with expected positive results, and showed a 95% match with a Kappa coefficient of 0.88. This ELISA Kit was adopted in the routine neonatal screening in filter paper. Of the 13,011 samples of total blood on filter paper, from different regions of Brazil, between January and December 2014, 12,968 (99, 66 %) were not reactive and 43 (0.33 %) were reactive. We conclude that the ELISA kit ETI-CYTOK-M reverse PLUS Diasorin, with modified protocol, for qualitative determination of IgM antibodies for CMV in blood collected on filter paper, is appropriate as an initial method of research for CMV infection in neonatal screening.

B-115

Influenza Virus in Long-Term Care Facilities

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Background: Influenza is an infection caused by influenza virus; they are three types A, B, and C; both A and B can be responsible for an epidemic disease. Influenza is a serious condition in Log-Term care Facilities where most of the residents are elderly, frail, disable, and are on multiple medications. Preventing transmission of influenza include: vaccination, testing, infection control, and appropriate treatment.

Design: 1,545 nasal swabs were collected from residents in Long-Term Care Facilities from November 2014 to February 2015. Tests were done using Binax rapid influenza diagnostic test (RIDT); the assay is an immunochromatographic membrane assay that uses highly sensitive monoclonal antibodies to detect influenza type A and B nucleoprotein antigens in respiratory specimens; the assay has 83% sensitivity and 96% specificity for influenza A, and 69% sensitivity and 100% specificity for influenza B. Statistical analysis was done using Analyse-it.

Results: 148 swabs were positive for influenza A and 5 swabs were positive for influenza B; we notice an increase in percentage positive test over the period tested with the highest prevalence in February. Only one patient needed hospitalization and no dearth has been reported.

	November	December	January	February
Total	6	224	926	389
Influenza A	0.0%	8.5%	9.3%	11.1%
Influenza B	0.0%	0.0%	0.4%	0.3%

Conclusion: influenza activity has been noted since December; the increase in the percentage positive results were higher in February may be due to testing patient with suspected influenza whereas in January it was done to screen the residents when one patient is presenting with influenza-like symptoms. Residents in Long-Term care Facilities has lower hospitalization and complication compared to national published data; which might be due to the higher vaccination rate to all residents and workers against the flu every year, early detection and administration of antiviral chemoprophylaxis to all residents as soon as they have an outbreak will prevent the complication, and the availability of infection control to prevent the spread of the illness.