Wednesday, August 1, 2018

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

Infectious Disease

B-046

Prevalence of Hepatitis B Surface Antigen Among Apparently Healthy Individuals in Ibadan Nigeria

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Background
Hepatitis B Virus (HBV) screen-and-treat programme targeting the general population is a pragmatic public health intervention. Preventive strategies are reasonable options in resource-limited countries. This study was aimed at evaluating the prevalence of HBV in Ibadan a cosmopolitan city, and to provide baseline data for onward intervention and treatment.
Methods
The study was cross-sectional. A total of 178 participants (118 females and 60 males) apparently healthy aged 10-60 years participated in the study. Socio-Demographic characteristics and risk factors for HBV infection were documented in a pretest structured questionnaire. Blood samples were obtained for qualitative detection of HBsAg using rapid chromatographic immunoassays with test kits from Micro Point (China) having sensitivity, specificity and accuracy of >99%, 97% and 98.8% respectively. Data was analyzed using Chi square.
Results
Prevalence of 6.2% was observed in the study population. HBV positivity of 4% was observed among the participants in the age range 41-50years and 0.6% among adolescent 11-20years. A prevalence of 3.4% and 2.8% were reported for females and males respectively.
Conclusions
High prevalence of asymptomatic HBV infection was observed among the adult population. Female gender had a higher spread of HBV and may have negative impact on the younger population if not treated because they are care givers. Population target screening, treatment and public health enlightment will benefit the population and also reduce the socioeconomic implication of HBV
Key Words: Asymptomatic Hepatitis B Virus, Prevalence, Ibadan

B-047

Development of a Bartonella henselae specific Human IgG ELISA

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1. Introduction *Bartonella henselae* causes cat scratch disease (CSD), an often self-limiting lymphadenitis in immunocompetent patients, and several other clinical entities. While cats are the natural reservoir for *B. henselae,* the pathogen is transmitted by cats, cat fleas and eventually by other arthropods. The clinical symptoms underlying CSD might be similar to those being suspicious for malignant tumors. Thus, an easy and reliable test for *B. henselae* infections is highly desirable. **2. Objective** The aim of this study is to design an ELISA for detection of *B. henselae* to improve the shortcomings of the currently used immunofluorescent test (IFT), e.g., objective and reproducible results and less hands-on time. **3. Material and Methods** Test development is based on different *B. henselae* strains and quality assured patient sera [(a)sera positively tested for anti *B. henselae* antibodies via IFT, (b) patients with typical symptoms, (c) sera of patients with PCR-based infection diagnosis]. Antigens were separated by ion exchange chromatography and fractions examined in lineblots. Potential fractions were further tested and optimized for ELISA. **4. Results** Patients with *B. henselae* infections show different patterns of antibody expression in western blots. Thus, there is obviously no universally usable antigen for diagnosis detectable. Crude antigen preparations (liquid grown or with cell culture) are not working reliably as they do not react with numerous patient sera. However, our tests show that there are certain protein fractions from *B. henselae* which react reliably and results from lineblots were successfully transferred to an ELISA-format with sufficient sensitivity. **5. Conclusion** We show a strategy for antigen testing and selection from *B. henselae* protein preparations for ELISA-based serology. Further processing of antigens is under investigation so that in future an ELISA for *B. henselae* is possible. **Funding** This study is financed by the state Hesse within the LOEWE III project.

B-048

Evaluation of individual and combined markers of urine dipstick parameters and total lymphocyte count as a substitute for CD4 count among HIV infected patients in resource-limited communities in Ghana

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Background: The diagnosis of HIV infections is based on CD4 count. However, most developing countries lack availability of CD4 count machines, reagents and expertise. The need to develop a less expensive and readily available diagnostic approach is warranted. We evaluated the individual and combined levels of urine dipstick findings and total lymphocyte count (TLC) as surrogate markers for CD4 count in a low-resourced community in Ghana. **Methods:** This cross-sectional study recruited 200 HIV infected patients from the Saint Francis Xavier Hospital, Assin Fosu, Ghana. CD4 counts, complete blood count (CBC) and dipstick urinalysis were measured for all participants. The threshold values were determined as <350 cells/μl for CD4 (new WHO criteria for starting HAART), <1200 cells/μl for TLC and ≥+ on urine dipstick analysis. CD4 T lymphocytes count was determined using the Becton Dickinson (BD) FASCount system, CBC was analysed using a five (5)-part automated blood analyzer (HORIBA Yumizen H500, Japan) and urinalysis was performed using dipstick urinalysis strips (Accu-Tell, ABT-UM-A33). Other signs of active infections and conditions that may interfere with urine dipstick analysis were also excluded. Receivers operating characteristic (ROC) curve was performed on the markers to obtain sensitivity, specificity, area under the curve (AUC), positive predicted values (PPV) and negative predicted values (NPV) were performed. **Results:** The mean age of participants was 43.09years. Proteinuria≥+ [(aOR=4.30(3.0 to 18.5)], leukocyturia ≥+ [aOR=2.91(1.33 to 12.5)], hematuria ≥+ [(aOR=2.30(1.08 to 9.64)] and TLC <1200 cells/μl [aOR=3.26(3.94 to 15.29)] were significantly associated with increased risk of CD4 count <350 cells/μl respectively. Using the individual markers, the best substitute marker for predicting CD4 count <350 cells/μl was proteinuria at a cut-off point ≥2++, AUC of 0.973, sensitivity of 97.6%, specificity of 100.0%, PPV of 100.0% and NPV of 89.1%. A combination of ≤1200 TLC + ≥2++ (leukoctyuria + proteinuria + hematuria) yielded an AUC of 0.980, sensitivity (72.8%), specificity (100.0%), PPV (100.0%) and NPV (97.9%). **Conclusion:** Proteinuria could serve as an early non-invasive screening tool for identifying HIV infected individual, but the combination of proteinuria, leukocyturia, hematuria and TLC serves as a better substitute marker for CD4 count in monitoring the disease progression among HIV patients in resource-limited communities.

B-049

Cost Savings from Appropriate Utilization of Procalcitonin (PCT) in an Acute Hospital

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Background: Procalcitonin has clinical utility in the initial diagnosis and subsequent management of patients with sepsis, particularly in guiding antibiotic therapy. Inappropriate utilization of this test adds unnecessary costs to patients without a concomitant improvement in patient outcomes. Khoo Teck Puat Hospital is a 700-bedded acute hospital with an Emergency Department and Intensive Care Units. **Objective:** We describe our laboratory's experience in facilitating appropriate usage for procalcitonin and measuring cost savings. **Methods:** As part of a resource utilization review, we tracked the number of procalcitonin requests in our hospital over a four-year period from January 2014 to December 2017 using the Laboratory Information System (LIS). The data was analysed, and feedback given to high usage wards such as surgical intensive care units. Topics on resource utilization and the role of procalcitonin were also raised during clinical-pathological conferences, departmental meetings, with active involvement of infectious disease physicians and anti-microbial stewardship pharmacists. **Results:** From 2014 to 2017, a total of 69,101 procalcitonin results were reported. The number of procalcitonin requests fell from 22,122 in 2014 to 12,533 in 2017 (43% reduction). The was a 14% to 19% year-on-year reduction in the total number of procalcitonin requests. The mean number of requests per patient-visit

fell from 1.76 to 1.68, 1.53, 1.44 from 2014 to 2017 respectively. The number of emergency department consults and admission numbers remained constant during this period, suggesting the fall was attributable to more judicious use in procalcitonin, rather than a drop in patient load. The number of patients who had a large number of procalcitonin requests also decreased. In 2014, there were 7 visits with more than 30 procalcitonins ordered. In 2017, no visits exceeded 30 procalcitonins. The highest number of procalcitonin ordered in a single visit was 28 in 2017. Overall, the percentage of visits with more than 10 procalcitonin decreased over the years and were 1.05%, 1.02%, 0.73% and 0.56% for 2014, 2015, 2016 and 2017 respectively ($p<0.001$). This represents a reduction of 700 procalcitonin requests per year among high usage patients, translating to a cost savings of SGD 52,500 per year. Among visits with procalcitonin requests, the percentage of patients who had more than one procalcitonin decreased from 30% to 25%, 23% and 20% from 2014 to 2015, 2016, 2017 respectively. Among patients who had multiple sets, the mean retesting interval appropriately increased from 2.84 to 2.83, 2.99 and 3.28 days from 2014 to 2017 respectively. **Conclusion:** While the repertoire and costs of laboratory tests continue to increase, our review shows that the clinical laboratory plays a key role in resource utilization. Active surveillance, collaboration with clinicians, providing regular and objective feedback to physicians, may alter ordering behavior and contribute to cost effective care.

B-050

PREVALENCE OF HEPATITIS B VIRAL INFECTION IN APPARENTLY HEALTHY ADULT PATIENTS OF PUBLIC PRIVATE PARTNERSHIP LABORATORY, UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA.

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Background: Hepatitis B viral infection is a chronic infection which could lead to chronic liver disease and in turn eventually leads to several other clinical outcomes associated with chronic liver disease. This study was carried out to determine the prevalence of hepatitis B viral infection within a period of eight month at the laboratory with the highest number of clients in the University college Hospital, South western part of Nigeria. **Materials and Methods:** The laboratory records of hepatitis B viral infection in apparently healthy adults, from July 2016 to March 2017 of our laboratory was compiled. Hepatitis B surface antigen (HBsAg) was assayed using electrochemiluminescence immunoassay "ÉCLIA" method on Cobas E immunoassay analyser, making use of sandwish test principle. Levels 1 and 2 quality control material specific for HBsAg produced by Roche was always included in our daily work. Any result less than 0.9 Col was considered non reactive while results between 0.9 to 1.0 were considered borderline results which were repeated for confirmation. Any result greater than 1.0 was considered reactive. **Results:** A total number of 503 apparently healthy adult patients were investigated. 288 (57.3%) of the population were male while 215 (42.7%) of the population were female. A total of 108 (21.5%) of the overall population tested positive to HBsAg. Of the 21.5%, 15.3% were male while the remaining 6.2% were female. This suggested a higher prevalence of HBsAg in men than in women. **Conclusion:** A decline in the level of hepatitis B virus infection could be achieved through public enlightenment campaign, massive immunization of children and adults who are at risk. Effective diagnosis, treatment and follow-up should be provided for those already infected.

B-051

Incidence of reactive HIV results during 2017 in private lab and oficials statistics

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Background: Since the 80s, Brazil has instituted as state politic, a Public Healthcare System that, in essence, values the universal, equal and integral acess. A country of continental dimensions, with an estimated population of 207.7 million inhabitants in 2017 and demographic pyramid tending to a populational senility, Brazil proposes to maintain healthcare services in increasing prices. Nevertheless, in practice, it can not hold a high level service. In the other hand, some islands of excellence persists, and are world references - amonsgst them, the STI/AIDS and Viral Hepatitis from the Ministry of Health. In this aspect, even with educatioal campaigns, and the acess to prevention methods, it can be verified the incidence increase of cases in the younger population. **Methods:** The HIV infection diagnosis, in Brazil, is governed by an especific guideline that determins the use of, at least, one of the Six Flow Chart. In our Lab, it has been used the Sixth Flow Chart. It consists in a fourth generation screening test (Abbott microparticles immunoassay by chemiminescence for the qualitative and simultaneous detection of anti-HIV 1 antibodies - M and O Groups- and/or Type Two, and the HIV p24 antibody in human serum and plasma), also, the Western blot confirmatory test (New Lab Blot BIORAD). The last, detects the multiple antibodies against each one os th viral proteins. In the study, it has been used our database from 2017, in which it was made 58,921 HIV screening tests. The reactive results incidence of 0.17% in total was stratified by age range and gender. **Results:** In the populational stratification, it was possible to observe some outstanding characteristics. 74.87% of the patients submitted to HIV test were female, and 96.80% of them were between 18 and 69 years old. The reactive results incidence, without segmentation, was 0.17%. Young men between 18 and 39 years old presented a amount of reactive result three times superior than the total population (0.57%). Lastly, men between 50 and 85 years old demonstrated an incidence two times superior than the population (0.41%). **Conclusion:** Female patients are the majority that submit to HIV test. One of the reasons is the fact that it is required in Prenatal Exams. In addition, another reason is that usually men neglect healthcare services, becoming a populational blind spot. Moreover, without generalizing, UNAIDS studies point to an active sexual life more and more precocious, without the use of preservative and the multiple partners. This facts illustrate the faces of men behavior, and corroborate to an elevated reactive HIV results incidence in young people. Also, the advent of erectile dysfunction medicines increases the incidence in over 50-years-old men. Thus, women are mostly affected as a consequence of these practices. **References:**1. Secretaria de Vigilância em Saúde. *Manual técnico para diagnóstico da infecção pelo HIV*. Ministry of Health, Third Edition, Brasília-DF, 2016.2. *HIV Prevention in the Spotlight. An analysis from the perspective of the Health Sector in Latim America and Caribbean*. UNAIDS, 2017.

B-052

Incidence Of Sorological Diagnosis Of Zika Viruses In Young Women During The Year 2017 In Private Laboratory And The Relationship With Sequels In Newborns

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Background: The Zika virus is an arbovirus, from the genus Flavivirus, belonging to the same group as Yellow Fever, Dengue, Japanese Encephalitis virus, among others. Discovered in 1947, it was isolated from monkeys samples, in Uganda (East Africa), through a monitoring network of wild yellow fever in the Zika Forest. In April 2015, the first case of autochthonous transmission was confirmed in Brazil. It was a new aggravation for pregnant women, and defined by scientific consensus, as one of the viral causes of congenital microcephaly, with possible links with other neurological disorders, besides cases reported by association with Guillain Barré Syndrome. Data from the Ministry of Health of Brazil indicate 3,037 cases of growth and developmental changes from November 2015 to December 2017, associated with infection by Zika Virus. **Methods:** In our service, we use the ELISA test (EUROIMMUN) for the indirect detection of the virus. It is based in the research of IgM antibodies. The techiques of Molecular Biology are also widely used, where the PCR (Polymerase Chain Reaction) identifies the viral DNA in samples. The use of rapid tests, according to the doctor discretion, is also a rapid and highly sensitive diagnostic tool, through immunochromatography by qualitatively detecting specific immunoglobulins. In this study, we used our database from the year of 2017, in which we performed 1,077 IgM serologies for Zika Virus, with an incidence of reagent results for Zika of 1.02% in total and stratified by age and sex. **Results:** In the populational stratification, it was possible to observe some striking characteristics, 64.62% of the pacients submited to the serology for the Zika Virus were female, 96.98% of them were between 18 and 49 years old, the age group with the highest prevalence of pregnancies. In women from 18 to 49 years old, the incidence of positive results was 1.19%, slightly above the total incidence. In men, there were only 3 cases of reactive results in 381 tests performed. **Conclusion:** Zika Virus infection in pregnant women is a serious public health problem in Brazil due to the correlation between infection and important sequelae in newborns. Brazil is a country with an expressive territorial area, elevated population density and a tropical climate with high precipitation rates. Therefore, this facilitates the proliferation of the vector Aedes Aegypti, requiring indistinct involvement among all to face this adversity. Consequently, the Ministry of Health should promote public policies to prevent new cases, besides epidemiological data and generation of scientific knowledge.

References:

Epidemiological Bulletin. *Febre pelo Vírus Zika: uma revisão narrativa sobre a doença*. Volume 46, #26, 2015. Secretaria de Vigilância da saúde- Ministry of Health. ISSN 2358-9450.

B-053

Changing from a modified to an unmodified testing of Hepatitis C Viral Loads: An evaluation of the Roche Cobas 4800 system Cobas HCV kit.

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Introduction
Hepatitis C virus (HCV) is one of the several viruses known to cause viral hepatitis. Nucleic acid testing (NAT) is a widely accepted confirmatory method to determine HCV infection. Assessment of HCV viral load provides better clinical utility in measuring baseline viraemia and improving the efficacy of antiretroviral treatment. As part of a response-guided therapy, a periodic assessment of HCV at specific intervals would allow further personalize treatment plan to become possible. A comparison study was done between the Cobas HCV (Roche Diagnostics, Switzerland) assay using the Cobas 4800 system, a complete and automated assay, against our current method, the Cobas TaqMan48 HCV v2 with sample preparation performed on the Qiagen EZ1 (Qiagen, Netherlands).
Material and methods
A total of 56 cell-free anonymized serum and plasma samples, with HCV viral loads ranging from undetected to Log^{10} 7.22 IU/mL, were tested on the Cobas 4800 system. Precision studies were derived from quality control materials at HCV viral loads of Log^{10} 2.38 and Log^{10} 6.36IU/mL. The data was assessed quantitatively using regression analysis and Bland-Altman bias plots. Qualitative assessment was made using a binary matrix to derive specificity and sensitivity of the assay. The limitation of the detection was derived from serial dilution of known high titer samples with negative serum.
Result
The Cobas 4800 HCV assay produced diagnostic sensitivity and specificity of 100% compared to the TaqMan48 method. Regression analysis showed a correlation of 0.8722x + 0.8805 (R=0.9697). A (-) Log^{10} 0.33 difference between the two method means. Precision studies based on the manufacturer's quality control material gave a standard deviation 0.06 (HCV; Log^{10} 2.38IU/mL) and 0.05 (HCV; Log^{10} 6.36 IU/mL). Limit of detection was determined to be 16.1 IU/mL, which was slightly higher than the manufacturer's declaration of 15.0 IU/mL.
Conclusion
The performance of the Cobas HCV assay on the Roche Cobas 4800 system was comparable to the current Cobas TaqMan HCV v2 method. Accuracy of viral load determination, instrument precision and performance limits were indistinguishable between both methods. However, the current semi-automated modified method of sample preparation on the Qiagen EZ1 and manual pre-PCR preparation for the TaqMan48 is prone to contamination and mistakes can occur. Almost throughout the whole process, operator involvement is required and subject to inter-operator variation. By adopting the automated and unmodified Cobas 4800 assay, operator intervention is only required at the start of the process and to transfer the instrument-prepared PCR plate between instruments. This allows a significant amount of walk-away time while maintaining high standards of quality and better utilization of operator skills for more complex operations.

B-054

Temporal patterns of troponin I and Jarisch-Herxheimer reaction in *Cryptococcus gattii* infection

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Background: Over the past two decades the fungal pathogen *Cryptococcus gattii* has emerged as a cause of disease in humans and animals in the Pacific Northwest. We report on a case of *C. gattii* infection in Skagit County, WA complicated by Jarisch-Herxheimer (JHR) reaction and increased cardiac troponin. **Case Report:** A 46-year-old woman was seen at a local hospital with febrile illness. Past medical history was unremarkable. She had an increased heart rate and blood pressure. A urinalysis was within normal limits. Chemistry showed elevated renal (BUN = 35 mg/dL; creatinine = 1.2 mg/dL) and liver (AST = 125 U/L; ALT = 180 U/L) enzymes, high glucose (154 mg/dL) and normal electrolytes. The patient was non-reactive for acute hepatitis panel and HIV. A base troponin I was normal (0.04 ng/mL). Hematology showed leukocytosis (25,000/mm3) and thrombocytopenia (98/mm3). A cryptococcal antigen assay (Cr-A) on serum was positive. A semi-quantitative analysis returned a 1:80 titer. A working diagnosis of cryptococcosis was made. A computed tomography (CT) scan of her chest showed a 50-mm lesion in the upper-right field of her lung. Lung biopsy pathology showed scattered fungal spores and positive periodic acid-Schiff (PAS) staining. A lumbar puncture to rule out asymptomatic CNS involvement returned a negative Cr-A on CSF. Fluconazole therapy was started but a clinical worsening of symptoms developed in 6 hours complicated by J-HR - temperature, blood pressure and platelet count decreased rapidly. At 12 hours chest and low back pain developed, and a troponin was 0.16 ng/mL. At 14 hours a second troponin had increased to 0.24 ng/mL. The patient was transferred to the ICU unit. Over next 96 hours the thrombocytopenia improved (112/mm3), troponin levels normalized (less than 0.07 ng/mL), and febrile illness and angina resolved. Fourteen days after onset of the illness, the patient's hematology and troponin were within normal reference range, the triage of transient events related to the JHR were absent. The Cr-A was still positive and returned a titer of 1:320. For the next 16 months the liver enzymes (AST,ALT) remained slightly elevated throughout the course of antifungal treatment. At 18 months post treatment the Cr-A was negative and the patient's liver enzymes normalized soon after therapy was halted. **Conclusion:** The JHR is a well-known complication of antimicrobial but not antifungal therapies. Studies have shown that cytokines, namely tumor necrosis factor and interleukins appear in the circulation transiently and correlate with symptom severity in pathogenesis of cryptococcosis. Antibodies against inflammatory cytokines have been shown to decrease the JHR. Sepsis that results from the presence of infectious organisms is frequently associated with changes in these inflammatory mediators. Elevations in cardiac troponin in patients with sepsis is common. The potential causes of troponin release during sepsis include decreased cardiac integrity, fungal polysaccharide capsule destruction and thrombotic dysfunction. This is the first reported occurrence of elevated troponin and a JHR reaction associated with antifungal treatment for cryptococcal disease.

B-055

Development of non-amplification DNA detection method for MPB64 in *Mycobacterium tuberculosis* complex

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Background: Even though the definite diagnoses for many diseases have been long believed to be performed with PCR, there are many issues to be solved in PCR. For example, they are (1) non-specific or false positive amplifications, (2) volume limit for a target sample, (3) deactivation of enzymes used, (4) complicated techniques, and so forth. The non-specific or false positive amplifications occur due to an excess DNA input, long targets or contamination. A sample amount used is about 1 μL, showing that at least 1000 copies have to be included in a 1 mL volume. This low concentration brings the false negative data. The deactivation of enzymes leads the deterioration of amplification efficiency. Further, the PCR techniques are complicated even at present. Thus the placement method of PCR is strongly needed without DNA amplification. In the present study, we propose a new method for detection of nucleic acids without any amplification and detect the gene of MPB 64, a specific protein in *Mycobacterium tuberculosis* (TB) complex. Methods: We have developed a new method using a combination of hybridization and thio-NAD cycling. The cDNA probes linked with FITC hybridized to the target sequences in the MPB64 gene. The anti-FITC antibody linked with ALP was applied, and then a cycling reaction was conducted by a dehydrogenase (3α-hydroxysteroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androsterone phosphate). That is, our new method is referred to as "non-amplification nucleic acid detection method". The single strand, the double strand and the DNA plasmid were used for the MPB64 gene detection, whereas *Mycobacterium avium* and *Mycobacter*ium *intracellulare* were used for a non-TB control sample. Two or four probes were prepared for each strand. Results: We obtained that the limit of detection was 3.7×10^5 copies/assay (i.e., 4.2×10^3 copies/μL), and that the limit of determination was 1.3×10^6 copies/assay (i.e., 1.4×10^4 copies/μL) for the single strand of MPB64. Using the double strand, the limit of detection was 1.3×10^6 copies/assay (i.e., 1.4×10^4 copies/μL), and the limit of determination was 4.2×10^6 copies/assay (i.e., 4.7×10^4 copies/μL). Using the plasmid, the limit of detection was 7.0×10^5 copies/assay (i.e., 7.8×10^3 copies/μL), and the limit of determination was 2.3×10^6 copies/assay (i.e., 2.6×10^4 copies/μL). We did not observe any response to the non-TB control samples. Conclusion: Because the protocol of washout is included in our method and the measurement volume can be larger than PCR, the possibility of false positive or negative results is decreased. The deactivation of enzymes can be avoided within the condition as described above. Therefore, our new method overcomes every difficulty of PCR. Furthermore, we should add one comment on the comparison with the Interferon-Gamma Release Assays (IGRA). The cost of our detection method is much less than IGRA.

B-056

Analytical validation of real-time PCR assay for detection of West Nile virus: the benign of laboratory diagnosis

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Background: Since its discovery in 1937, the West Nile Virus (WNV) has expanded beyond its known geographical reach and caused disease in humans on several continents. It is currently the most common cause of neurological diseases caused by arboviruses worldwide. WNV is an arbovirus of the *Flaviviridae* family, which has its RNA genome of approximately 11,000 kb. Maintained in nature in an enzootic transmission cycle between birds and mosquitoes, WNV can also infect humans and other vertebrates. Such infections are usually mild or subclinical, with 80% of human infections being asymptomatic. In the diagnostic laboratory WNV can be inferred by ELISA, however, this assay is limited due to the difficulty in the differentiation between WNV and others virus. Molecular methodologies such as real-time PCR have been indicated since they are highly sensitive and specific for the detection of RNA viruses, including WNV. **Objective**: To describe the analytical validation of a real-time PCR assay for detection of West Nile Virus using commercial control. **Methods**: The primers and probes were designed from two conserved regions of the WNV genome (New York 1999 WNV isolate): 3' noncoding region (NCR) and envelope region (ENV). Performance of assay was evaluated using commercial quantified positive controls and the parameters of analyze included: (i) Determination of threshold (Dilutions of RNA which were run in triplicate); (ii) Analytical sensitivity (Limit of detection in replicates with concentration of RNA in the range of 1.250 to 19 copies/µL); (iii) Intrassay and interassay precision (Test of RNA in triplicates with one concentration at the limit of detection, one with a concentration 20% above the limit of detection, and one with a concentration 20% below on the same day and 3 different days); (iv) Analytical specificity (Interference study with Dengue virus, Chikungunya virus, Zika virus, Yellow fever virus); (v) Test of spike in (A negative sample was spiked with positive control). **Results**: The determination of the detection threshold remained within the range of linearity of a standard curve with a coefficient of variation (CV) of 0.99. The detection limits were 39 copies/µL for both the targets (95% confidence interval). The regression equations obtained show good amplification conditions with positive correlation between the variables, with a coefficient of determination (r^2) of 0.99 and amplification efficiency of 94% (NCR) and 103% (ENV). The experiments performed to evaluate the precision demonstrated optimal repeatability and reproducibility. No cross-reactivity was observed. The spiked sample presented positive results with a minimum value of 3.125 copies/mL of sample. **Conclusion**: This analytical validation provides data indicating that the specificity and sensitivity of the assay for a WNV detection system fulfilled the criteria requested by international guidelines. This study was not tested in real clinical samples therefore, before implementation of the assay in routine diagnostic laboratory; a clinical study is needed to establish the method in clinical and operational settings. Therefore, further studies will be performed to more effectively evaluate the possibility of using this method in routinely detection of WNV in various clinical samples.

B-057

Yellow Fever: what has been happening in Brazil?

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Background: Yellow Fever (YF) is a zoonotic flaviviral disease caused by the Yellow Fever virus (YFV), which is carried by the vector mosquitoes *Haemagogus* and *Sabethes* (sylvatic cycle) and *Aedes aegypti* (urban cycle). This disease is a reemerging, zoonotic, noncontagious viral hemorrhagic disease endemic and epidemic in tropical regions of South America and Africa to Africa and South America; outbreaks occasionally occur among human and nonhuman primates. According to the World Health Organization (WHO), YF remains an important public health problem and has been estimated at over 200,000 cases per year worldwide, causing 30,000 deaths. The true incidence of YF infection is unknown due to insufficient reporting and ground surveillance. Therefore, there is an urgent need to detect and study the prevalence of YFV in Brazil and regions in order to contribute to the implementation of public health policies in Brazil. **Objective**: To describe the behavior of Yelow Fever virus infection in Brazil and federative units during period of August 2017 to January 2018. **Methods**: This was a retrospective study, carried out through consultation of laboratory test results stored in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil) web LIS. All results of a Yellow Fever IgG and IgM obtained and released from August 2017 to January 2018 were compiled. Epidemiological data such as gender, age and region of the country of Yellow Fever IgG and IgM patients were statistically analyzed. **Results:** A total of 516 patients from all over the country were evaluated between 2017 and 2018. There was a predominance of patients from the Southeast region (75.4%), home of the laboratory, followed by the South (8.3%), Northeast (7.0%), Midwest (6.2%), and North (3.1%). The rates of positivity for Yellow Fever IgG and IgM were 25.8% and 2.7%, respectively. Among IgM positive cases, 7.1% were children (under 20 years), 92.9% adults (between 20 and 60 years). There was a male predominance in adults and female predominance in children patients. 92.9% of these cases were from Southeast region, and 7.1% were from Midwest. In Southeast, serology was mostly positive in men while in the Midwest, the positivity was higher among women. Considering just January 2018 the number of positive IgM cases was 3.67% more than all cases of the period analyzed of 2017. **Conclusions**: The high levels of positive IgG antibody may be attributed to vaccine impact, therefore the data of IgG antibody was not detailed in this study. The epidemiological profile of seropositive for YF IgG and IgM antibodies assisted by IHP was similar to the Ministry of Health regarding the Brazilian population. A complex combination of ecological, social, and behavioural factors may help to explain the severity and efficient spread of the YFV in Southeast Brazil, particularly its dissemination to the Atlantic coast. Despite being an area where routine immunisation is recommended and there is good vaccination coverage in the young population, the epidemic reached and hit with severity the rural area, due to poor vaccine coverage among adults.

B-058

Comparison of two processing methods to traditional nucleic acid extraction for qPCR in faecal samples

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Background: Gastrointestinal infections cause a huge impact on world health. Real-Time PCR (qPCR) has become a routine and robust technique for improving the diagnosis. Commercial extraction procedures for obtain nucleic acid (NA) necessary to carry out the amplification are usually expensive, time consuming and utilize dangerous reagents for human health. This study evaluated two quick extraction processes in faecal specimens to improve a rapid diagnosis in combination with qPCR. **Methods**: This study was carried out in 78 faecal samples from patients with clinical suspicion of gastrointestinal disease (collected in July 2017). NA was isolated with three different types of extraction procedures. The reference standard (RS) was a commercially available kit using a silica-based matrix, VIASURE RNA-DNA Extraction Kit (Certest Biotec, Spain). Two quick processes were compared to RS, VIASURE Lysis Buffer (LB) which has been recently developed by Certest, and transport medium compatible with PCR reagents MSwab™ (MS) from Copan (Italy). Both obtained NA using a simple and rapid boiling procedure. qPCR assays run on thermocycler Cobas Z480 (Roche Diagnosis) using VIASURE gastrointestinal panel (Certest). **Results**: A total of 111 pathogens (53 bacteria, 18 parasites and 40 viruses) in 73 positive samples were diagnosed. Co-infections were identified in 40 % total specimens. The difference Cq values (ΔCq) between RS and LB in bacteria and parasite were in average less than 3. For viruses, the difference was between 3-6. 8 positive samples were no detected with LB procedure, 2 of them were considered random positives. In regard to MS, ΔCq compared with RS in bacteria and parasite was in average ≥3. For viruses, in almost all cases were considerably greater than 3. In Astrovirus and Norovirus GII the difference was >10 and Norovirus GI positive samples were not detected. 23 positive samples were no identified with MS procedure, 8 random positives. **Conclusions**:

1- LB is a simple and rapid procedure, valid for universal NA isolation in fecal specimen and compatible with qPCR, which improve a rapid diagnosis.
2- In comparison with another quick treatment, LB identified most microorganism and the Cq values are closest to RS.

B-059

Comparison of HDV RNA level and severity of liver disease among subjects with HDV/HBV infection.

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Background: Hepatitis B virus(HBV) and hepatitis delta virus(HDV) superinfection often show severe chronic hepatitis, but sometimes it lacks any symptoms of liver disease in some patients. Mongolia is one of the countries with the highest HDV prevalence in the world. According to study conducted in 2015, 10.6% of apparently healthy Mongolian population tested positive for HBsAg and 61% of HBsAg positive subjects were positive for HDV-RNA. Another study by Japanese group found correlation between circulating HDV RNA level and liver injury. We assessed 46 subjects whose serum HBV DNA and HDV RNA levels were quantitated by RT-PCR to see if there is any correlation of HDV RNA level with the subjects' alanine aminotransferase (ALT) levels and their liver damage status (ASC-asymptomatic, CH-chronic hepatitis, LC-liver cirrhosis) diagnosed by doctors. **Methods:** We conducted a retrospective study to review records for 46 subjects (age 26-72, female 22, male 24) who were tested positive for both HDV RNA (Genesig, UK) and HBV DNA (Abbott, USA) at Gyals Medical Center in Mongolia, between 2016-2017. All data were analyzed by STATA statistical analysis software. **Results:**
There was no statistically significant correlation between the severity of liver damage and level of HDV RNA (**Figure 3**). However, subjects with LC diagnosis showed weak statistical correlation (p=0.088) between ALT level and HDV RNA level (**Figure 2**). When we compared HDV RNA levels with HBV DNA levels in ASC, CH and LC subjects, ASC subjects had low level of HBV replication (under LOD) and higher level of HDV RNA replication (**Figure 1**). The levels of HBV DNA in serum did not differ among the 3 groups. **Conclusion:** In this study, weak correlation was observed between serum HDV RNA level and ALT level among LC subjects and it could mean that HDV RNA may play role in liver pathogenesis, as confirmed by previous studies. An interesting trend was seen among ASC subjects: low level of HBV replication was observed with higher level of HDV replication, which calls for broader study involving bigger subjects pool. **Figure 1.** Relationship between levels of HDV RNA (copies/ml) and of HBV DNA (IU/ml). ASC-asymptomatic carriers, CH-chronic hepatitis, LC-liver cirrhosis.**Figure 2.** Relationship between level of HDV RNA and level of alanine aminotransferase (ALT), in serum of liver cirrhosis (LC) subjects.**Figure 3.** Levels of HDV RNA in severity of liver disease. ASC-asymptomatic carriers, CH-chronic hepatitis, LC-liver cirrhosis.

B-060

Cytomegalovirus Quantitative Detection: a Novel, Rapid and Sensitive Ready-To-Use Real-Time PCR-Based Kit

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Background: Human Cytomegalovirus (CMV) is a ubiquitous human-specific DNA virus, belonging to the *Herpesviridae* family. CMV infection is usually asymptomatic and is common even in the general immune-competent population, with an infection rate of 50-80%. In immunocompromised patients that undergo transplantation the CMV infection rate is even higher, being an important cause of morbidity and mortality. Congenital CMV infection is the most common congenital infection worldwide and is the leading non-genetic cause of sensorineural hearing loss in children. Respect to traditional techniques of virus isolation, molecular methods demonstrated to be a rapid and sensitive alternative for virus detection. The aim of this work was to evaluate the performance of a new quantitative freeze-dried and ready-to-use assay designed to detect CMV DNA in human samples. **Methods:** A novel quantitative Real-Time PCR based-assay was developed as a ready-to-use test with specific sets of primers and probes able to amplify two different conserved regions within CMV genome. A third set of primers and probe, specific for a Human Beta Globin gene fragment, was used as an internal control. These three sets were combined in a lyophilized ready-to-use mix and all the targets were co-amplified and detected using different Real-Time PCR instruments. In the present study, several samples obtained from San Raffaele Hospital in Milan and previously diagnosed as positives and negatives with the "CMV ELITe MGB® Kit" (ELITech Group) were investigated. Real-Time PCR reactions were performed using DNA extracted from plasma, swab, bronchoalveolar lavage or biopsy. **Results:** This new quantitative freeze-dried ready-to-use assay showed to be specific for CMV, giving robust and accurate amplification of CMV target regions with a sensitivity and a specificity of 100%. All the tests performed with this assay confirmed the results obtained at San Raffaele Hospital and indicated a Limit of Detection below 10 genome copies per reaction, thus reaching the same LoD of CMV ELITe MGB® Kit. **Conclusion:** This novel Real-Time PCR assay proved its effectiveness for the quantitative detection of CMV DNA in clinical samples. Its high-sensitivity and specificity, associated with the ready-to-use feature and room temperature storage, would easily improve the early and correct management of CMV-affected patients.

B-061

A compact PCR system for rapid and sensitive detection of Middle East respiratory syndrome-coronavirus

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Middle East respiratory syndrome (MERS) is a zoonotic viral respiratory disease with dromedary camels as the major reservoirs. Rapid identification of the etiological agent, MERS-coronavirus (MERS-CoV), near patients could greatly facilitate efficient disease management and control. The POCKIT™ COMBO system (GeneReach), including a compact automatic nucleic acid extraction device (taco™ mini) and a simple insulated isothermal PCR device (POCKIT™), enables pathogen detection at settings close to points of need. Clinical performance of a qualitative matrix MERS-CoV RT-PCR method targeting both *upE* and *ORF1a* marker genes (LoD95%, 30 and 17 genome equivalents, respectively) on the POCKIT™ system were evaluated. Clinical performance of the index matrix RT-PCR was compared to a commercial real-time matrix RT-PCR (RT-qPCR) targeting the same genes (RealStar® MERS-CoV RT-PCR Kit; Altona Diagnostics) on a RotorGene system (Qiagen) by testing 102 nasal swab samples. Positive results were derived from positive detection by one of the two markers. Nucleic acids extracted by MagNA Pure system (Roche) or taco™ (GeneReach) were tested by the two RT-PCR methods in parallel. 2x2 contingency analysis of the results shows that 40 were positive and 58 negative in both methods, while one was reference RT-qPCR negative/index RT-PCR positive and three were reference RT-qPCR positive/RT-PCR nagative. Interrater agreement calculated by kappa test was 96.08% (CI95%, 91.62 - 100%; κ = 0.92), indicating that the index matrix RT-PCR and the reference RT-qPCR had excellent agreement. Components of the POCKIT™ Combo system have also been integrated into an automatated sample-to-results device to help reduce hands-on time and enhance test consistency. The reagent is ready in a lyophilized format. Providing detection performance comparable to laboratory real-time RT-PCR system, the MERS-CoV RT-PCR on the POCKIT™ systems have potential to serve as an effective point-of-need tool for rapid detection of MERS-CoV.

Table 1. 2x2 contingency analysis: comparison between reference real-time RT-PCR system and index RT-PCR on the POCKIT™ system

		MERS-CoV real-time RT-PCR		
		Positive	Negative	Total
MERS-CoV RT-PCR (POCKIT™ system)	Positive	40	1	41
	Negative	3	58	61
	Total	43	59	102

Agreement, 96.08%; CI95%, 91.62 ~ 100%

B-062

New diagnostic markers for differentiation of acute and convalescent human cytomegalovirus infections

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Background
Human cytomegalovirus (HCMV) is the most common viral pathogen of congenital infections. The risk of virus transmission from mother to foetus is highest in acute primary infection during pregnancy. In affected children, HCMV can cause severe complications. Serological diagnosis of early primary infections, however, is challenging for two reasons: 1) presence of anti-HCMV IgG is indicative for primary infection only if seronegativity has been documented in a previous sample; 2) an-

ti-HCMV IgM antibodies may persist and can be associated with different clinical settings, such as acute primary, convalescent primary or recurrent infection. Here, we present two novel markers, anti-HCMV p52 IgM and anti-HCMV gB IgG, for better differentiation of acute and convalescent phases of HCMV infection.
Material & Methods
A commercial panel for anti-HCMV seroconversion (Biomex, Germany) comprising 25 follow-up samples covering 124 days from a male was analysed. In addition two patients from a reference laboratory (Lübeck) were tested: Patient 1 presented with fever and fatigue; serum samples were taken one, two, four, five and seven months after onset of symptoms. Patient 2 is a female whose serum samples were taken 20 months before and during pregnancy (week 8 and 33). Samples were tested for anti-HCMV IgM and IgG as well as anti-HCMV p52 IgM and anti-HCMV gB IgG using ELISA (Euroimmun AG, Germany). For detection of anti-HCMV p52 IgM and anti-HCMV gB IgG, new recombinant antigenic substrates are applied. ELISA were conducted according to manufacturer's instruction.
Results
The commercial panel demonstrated seroconversion of anti-HCMV IgM and IgG around day 35 of monitoring as well as positivity for anti-HCMV p52 IgM between day 35 and day 85. When anti-HCMV p52 IgM declined below cut-off, anti-HCMV gB IgG appeared. Patient 1 revealed high titer anti-HCMV IgG in all samples. Anti-HCMV IgM and anti-HCMV p52 IgM were initially present but turned negative three to four months after onset of symptoms. At that time, seroconversion of anti-HCMV gB IgG was observed. Patient 2 was tested negative in all assays before pregnancy, but showed high titer anti-HCMV IgG and anti-HCMV gB IgG, when she was eight weeks pregnant. Anti-HCMV IgM and anti-HCMV p52 IgM were negative. Equal results were obtained in pregnancy week 33.
Conclusion/Discussion
The antibody courses in the commercial panel and patient 1 support the concept of anti-HCMV p52 IgM being a putative marker for acute HCMV infection, while detection of anti-HCMV gB IgG is indicative of a convalescent phase. IgG seroconversion of patient 2 implies that the woman got infected with HCMV. However, according to the concept, infection is likely to be convalescent at the time of second blood withdrawal (anti-HCMV p52 IgM negative, anti-HCMV gB IgG positive) and thus, the risk of virus transmission to the eight weeks old foetus would be low.

B-063

The time course of calprotectin release from human neutrophil granulocytes after challenge with E. coli

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Background: Plasma calprotectin has previously been reported as a promising biomarker for sepsis. Calprotectin is released from neutrophil granulocytes upon inflammatory activation. However, little is known about how promptly calprotectin is released from these cells in response to encounters with bacteria or pathogen associated molecular patterns (PAMP). A new turbidimetric method (PETIA) that offers a relatively inexpensive analysis of calprotectin with rapid turn-around times from sampling to laboratory results was launched last year. Rapid turn-around-time for analysis and early release of calprotectin upon inflammation and/or infection suggest that calprotectin can become a useful biomarker with widespread clinical use. The aim of the present study was to elucidate the kinetics of calprotectin release from blood neutrophils, exposed to E. coli. We also analyzed other inflammatory mediators with known kinetics as comparison, and kidney injury molecule 1 (KIM-1) as a non-blood cell inflammatory biomarker. **Methods:** Whole blood samples were exposed to E. coli bacteria in vitro. Blood samples were collected after 0, 1, 2, 3 and 4 hours. Plasma calprotectin was analyzed with a particle enhanced turbidimetric immunoassay (PETIA) while tumor necrosis alpha (TNF-α), interleukin-6 (IL-6), neutrophil gelatinase-associated lipocalin (NGAL) and KIM-1 were analyzed by ELISA. **Results:** When the blood cells were exposed to E. coli, the calprotectin levels began to increase within 1-2 hours after the exposure. IL-6, TNF-α and NGAL increased above baseline at 1 hour after bacterial exposure while levels of KIM-1 were beyond detection limit in almost all plasma samples. **Conclusion:** Our data demonstrated that calprotectin increases early in response to bacterial challenge. Given the logistic advantages of the calprotectin PETIA analysis, this biomarker may be of interest for early diagnosis of bacterial infections.

B-064

A brazilian case report of yellow fever infection

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Background: Yellow fever (YF) is a mosquito-borne viral hemorrhagic fever, which is a serious and potentially fatal disease with no specific antiviral treatment that can be prevented by an attenuated vaccine. Since December 2016, Brazil is affected by an unusually large and expanding yellow fever outbreak, with over 3,500 suspected cases reported and several hundred deaths. In early 2017, the Brazilian Ministry of Health reported outbreaks of this disease in several eastern states, including areas where yellow fever was not traditionally considered to be a risk. In January and February 2018, 88 cases of YF were recorded in the state of Minas Gerais, Brazil. Descriptive epidemiological evidence suggests that the outbreak so far shows a sylvatic transmission pattern with human infections being acquired from non-human primates (NHP) via forest-associated mosquito species. However, recent research has identified urban mosquito vectors to be competent for transmission of yellow fever virus (YFV), suggesting a risk of re-emergence of urban YF in Brazil. **Objective**: To report the laboratory profile of a brazilian patient with yellow fever infection which was confirmed by molecular method (Real Time PCR). **Methods**: The laboratory tests requested were carried out in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil according to care routine. **Results**: A 49-year-old man was admitted to a hospital in January 11, 2018 because of high fever with severe headaches, fatigue and weakness. Alpha 1 antitrypsin was within the normal range, Ceruloplasmin was significantly decreased, which may be indicative of hepatic degradation. Antibody Anti LKM-1, autoantibodies against Smooth Muscles (ASMA) and Cell Nuclei (ANA) were normal and Autoimmune liver disease and rheumatic diseases were excluded, respectively. Besides that autoantibodies against Granulocyte Cytoplasm (cANCA/pANCA) were normal and Wegener's granulomatosis, glomerulonephritis, primary sclerosing cholangitis, ulcerative colitis, Crohn's disease were also excluded. In the patient´s infectious examination, serologic laboratory tests (IgM antibody) of Anti Chikungunya, Anti Cytomegalovirus, Anti Dengue, Anti Epstein Barr, Anti Hepatitis A, B, C and E, Anti-Herpes Virus, Anti HIV, Anti Leishmaniasis, Anti Leptospirosis, Anti Parvovirus B19, Anti Zika Virus were negative. The clinical course presented fatal complications and the patient died 7 days of the onset of symptoms. C**onclusion**: The case reported refers to a patient with history of stay in the municipality of Brumadinho, state of Minas Gerais, Brazil, an area where the circulation of yellow fever virus is currently occurring. Moreover, the case has no history of yellow fever vaccination. Although Brazilian health authorities have swiftly implemented a series of public health measures in response to the outbreak, including mass vaccination campaigns, it may take some time to reach optimal coverage in these areas given the large number of susceptible individuals.

B-065

Development of a Comprehensive set of Zika Virus Reference Materials for Validation and Evaluation of Performance of Serological and Molecular Assays.

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Background: Outbreaks of Aedes mosquito borne Zika have occurred in areas of Africa, Southeast Asia, Pacific Islands, Brazil and continues to spread rapidly through many countries of the Americas. Zika virus causes the infectious Zika fever and has been linked to Guillain-Barre syndrome and neurological birth defects. In response to the outbreak, several serological and PCR-based Zika assays have been developed and approved under FDA's Emergency Use Authorization. Clinical laboratories and diagnostic test developers need robust positive reference materials and panels that can evaluate these assays across the entire reportable range for sensitivity and linearity. SeraCare has developed a suite of products that not only fulfills the need for specificity in current Zika assays that is critical in isolating the proper virus to determine treatment; but also provides safe to handle, virus-like material that can be used as positive control in Zika diagnostic assays. **Methods:** SeraCare has developed three new products; AccuPlex™ Zika RNA positive reference material, AccuSpan™ Zika RNA Linearity Panel and the AccuSet™ Zika IgM Performance Panel. AccuPlex Zika RNA Reference Material and AccuSpan Zika RNA Linearity Panel is formulated using SeraCare's recombinant virus technology and intended for use with nucleic acid test methods for external quality control that detect the ZIKV 2007 strain. The entire genomic RNA sequence is packaged into specially modified recombinant Alpha viral vectors and diluted in defibrinated human plasma (DHP). The AccuSpan

Zika RNA Linearity Panel consists of six positive members, each prepared from the preceding member by diluting recombinant Zika Virus with Zika RNA negative diluent in serial ten-fold dilutions beginning at member 1, the highest positive (target concentration of 1.0E+06 copies/mL verified by dPCR analysis), and ending with member 6, the lowest positive. The diluent was prepared from normal human defibrinated plasma negative for Zika RNA. AccuSet Zika IgM Performance Panel is a 10-member performance panel consisting of undiluted, naturally occurring undiluted plasma samples with reactivity ranging from negative to positive for Zika IgM and IgG antibodies. **Results:** AccuPlex Zika Reference Material is a ready-to-use product that is formulated for use with PCR- based assays that detect the ZIKV 2007 strain. The AccuSpan Zika RNA Linearity Panel can be used to evaluate the dynamic range of Zika RNA assays, identifying consistency over a linear range, verifying lot changes and performing linearity studies. AccuSet Zika IgM Performance Panel is a comprehensive panel consisting of real patient samples for serological test methods for Zika IgM and Zika IgG. The panel members have been highly characterized and comprehensively tested on several methodologies including the CDC algorithm and therefore designed to help researchers evaluate Zika infectivity from that of related flaviviruses. **Conclusions:** SeraCare has developed a suite of Zika products for PCR based and serological test methods that enables researchers and diagnostic manufacturers to expedite the development, evaluation and validation of Zika assays.

B-066

Phenotypic Susceptibility Profile of Methicillin Resistant *Staphylococcus aureus* in the Dominican Republic

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Background. Methicillin-resistant *Staphylococcus aureus* (MRSA) poses a major challenge to clinicians. The prevalence of MRSA has increased over the past decades. The burden can vary by geography and healthcare systems. There is a paucity of data on MRSA susceptibility patterns in developing nations. Local susceptibilities have an important role for the selection of empiric treatment choices in patients with suspected MRSA infections and in those with beta-lactam allergies. We seek to define the prevalence and resistance profile of SA in the Dominican Republic (DR). **Methods.** This is a retrospective review of resistance patterns of *S aureus* (SA) isolates from a clinical laboratory in the DR (Amadita Laboratories). Amadita provides services nationwide. Data collected from 2016-17 included organism sensitivity patterns and geographic location. Automated susceptibility testing (Vitek®2, bio-Mérieux) was used for susceptibilities and clindamycin inducible resistance testing. **Results.** Of 1674 samples of SA, 869 (52%) were MRSA and 805 (48%) were susceptible to methicillin (MSSA). MRSA resistance to tetracycline was high (82%). Clindamycin resistance was more likely to be inducible (19% vs 1.5%). Eight isolates were resistant to vancomycin (VRSA) and 29 isolates (3.3%) had minimum inhibitory concentrations above 2. MRSA was more common in rural areas (56% vs 50%). Vancomycin resistance was more common in urban areas (2.8% vs 0.2%). Antimicrobial susceptibilities are shown in Table 1.
Table 1. Antimicrobial resistance for SA isolates by drug resistance category (%)

	Ciprofloxacin	Clindamycin	Erythromycin	Gentamicin	Levofloxacin	Linezolid	Oxacillin	Quinoprustin/dalfopristin	Penicillin G	TMP-SMX	Rifampin	Tetracyclines	Tigecycline	Vancomycin
All isolates	11.7	18.9	63.9	3.4	2.8	1	52	0	95	2.9	0	45.5	0	1.2
MSSA	12.4	33	38.5	2.1	1.8	1	0	0	90	6	0	58	0	1.4
MRSA	11.1	5.7	87.5	4.7	3.7	1	100	0	99.5	0	0	82.2	0	0.9

Conclusion. In this nationwide sample, MRSA was more common than MSSA. SA resistance profiles in the DR have high rates of resistance to tetracyclines. Clindamycin resistance was higher for MSSA isolates and was commonly inducible. Clinical laboratories in the region should consider routine testing of inducible resistance to clindamycin. Trimethoprim-sulfamethoxazole (TMP-SMX) and linezolid have the most optimal susceptibility profile of available oral agents against MRSA. The rise of vancomycin resistance is concerning and requires further study.

B-067

Evaluation of a Procalcitonin Assay on the Atellica IM Analyzer

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Background: Procalcitonin (PCT) is a 116 amino acid peptide that shares a common structure with the prohormone of calcitonin. Under normal metabolic conditions, calcitonin prohormone is produced by the thyroid's C-cells, where it undergoes proteolysis to yield the hormone calcitonin. Calcitonin is then involved in calcium homeostasis. Under normal conditions, plasma levels of the calcitonin prohormone have been shown to be under 0.1 ng/mL. However, during episodes of severe bacterial infection and sepsis, the level of blood-circulating PCT increases to levels generally above 2 ng/mL. In response to proinflammatory stimuli, such as bacterial infection, operation, or trauma, PCT can be produced by nearly every tissue of the body. Siemens Healthineers has developed a procalcitonin assay for the Atellica® IM Analyzer with acceptable sensitivity, precision, and linearity to aid in the risk assessment of critically ill patients for progression to severe sepsis and septic shock on their first day of intensive care unit (ICU) admission. The Atellica IM PCT Assay is an 18-minute sandwich immunoassay with a range of 0.02 to 50.00 ng/mL, and is aligned to the BRAHMS KRYPTOR assay. **Method:** The Atellica IM PCT Assay's performance was assessed with two lots of reagents. Imprecision and functional sensitivity were evaluated using two levels of control materials, a panel of five human serum precision samples, and a panel of five human serum functional sensitivity samples containing low levels of PCT analyte, tested twice a day for 20 days for a total of 80 replicates on two instruments. Linearity studies were conducted using nine human serum samples equally spaced across the assay range in a known mathematical relationship and evaluated using two reagent lots. A method comparison to the BRAHMS KRYPTOR reference method was confirmed using one lot of reagents and 265 serum patient samples with known BRAHMS KRYPTOR values. **Results:** The data obtained with the Atellica IM PCT Assay demonstrated correlation to the BRAHMS KRYPTOR method, yielding a Passing-Bablok slope of 1.02 and regression coefficient of 0.98. A 20 day precision study yielded within-lab precision CVs between 2.1% and 13.7% for the two reagent lots using samples containing between ~0.03 ng/mL and ~20.65 ng/mL of procalcitonin. Functional sensitivity for both reagent lots was ≤0.04 ng/mL. Linearity studies demonstrated that the PCT assay is linear across the assay range of 0.02 to 50.00 ng/mL. **Conclusion:** The performance of the Atellica IM -PCT Assay has been assessed and the results show an accurate, sensitive and precise method for the measurement of Procalcitonin in human serum. The Atellica IM PCT Assay is in alignment with the BRAHMS PCT Sensitive Kryptor assay and may be a valuable tool in clinical laboratories for the accurate measurement of procalcitonin in human sera. Not available for sale in the U.S. Future availability cannot be guaranteed. **Siemens Healthcare Diagnostics**
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B-068

Optimization of the lamination system of urine samples in Flow Cytometry

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Background: In the iQ200 automated urine microscopy Analyzer a urine sample is sandwiched within a special fluid called "lamina", IQ lamina. This system is coupled to a digital video camera. The lamina and flowcell are key to hydrodynamically orienting the particles in the urine and increase the efficiency of cellular counters. For this lamination the reagent IQ lamina is used, with the need for a higher volume of this reagent the higher the turbidity of the sample;This study aimed to manage the urine sediment analysis routine and reduce the cost of the laminating reagent after understanding the system and measuring its performance using a graduated test tube to measure volumes used in the monthly and preventive calibration, the consumption of reagents according to the density or viscosity of the samples, either in continuous or intermittent flow, without compromising the quality of the result. **Methods:** A total of 167 turbid samples, 243 clear samples and 450 slightly turbid samples were selected during the months of March, April and May 2017. In order to eliminate the interference of amorphous particles, only non-amorphous urine samples were used. We note that the IQ 200 Sprint linearly processes up to 1000 cells / µL. We optimize a continuous flow of samples, in detriment to the intermittent flow, allowing a lower consumption of IQ lamina. **Results:** We observed a gradual reduction in the use of the IQ lamina reagent, with a reduction of 18 ml / day to 12.7 ml / day, below the target of 15 ml / day. With a saving in gallons of 0.7 / day, reaching 231.8 gallons / year. And an annual financial gain of $ 6,500

Conclusion: Managing the urine routine provided positive results, with reduced input and consequent financial gain, without compromising the quality of the exam. This was achieved by implanting a continuous stream of samples.

B-070

Ready-to-use Stabilized qPCR Assays for Detection of Zika, Dengue and Chikungunya Viruses

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Background:
Zika virus (ZIKV), Dengue virus (DENV) and Chikungunya virus (CHIKV) are arthropod-borne arboviruses transmitted by mosquitos of the *Aedes* genus. They cause similar clinical presentations, especially in the initial stages of infection, and so an early and accurate diagnosis is imperative. Polymerase chain reaction (PCR) based diagnosis has shown to be a sensitive and specific method for pathogen identification purposes. The cross-reactivity of the antibodies of these arboviruses limits the use of serology, so real time PCR is a detection method commonly used during the acute phase of the infection. Stabilization of molecular assays can overcome limitations associated with qPCR technique such us assay variability, risk of contamination and the need for cold-chain, thus enhancing the spread of qPCR technique.
Methods:
A retrospective study was performed on 66 samples from the External Quality Assesment (EQA) programs QCMD and INSTAND. Samples were collected from April 2014 to October 2017. Genomic RNA was isolated using "QIAamp Viral RNA Mini Kit" (Qiagen). Nucleic acids were analysed with two different lyophilised real-time PCR detection kits. 5 µl of sample were amplified with "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit" (Certest Biotec), while 25 µl of sample were necessary for "TaqMan Zika Virus Triplex kit (ZIKV/DENV/CHIKV)" (Thermofisher). Performance results of both kits were compared with the corresponding reports from EQA programs.
Results:
According to EQA programs reports, 12/66 samples were ZIKV positive, 24/66 were DENV positive and 19/66 were CHIKV positive. All samples were correctly detected with "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit". "TaqMan Zika Virus Triplex kit (ZIKV/DENV/CHIKV)" failed in the detection of two ZIKV and three CHIKV positive samples, while four unspecific amplifications for ZIKV and another one for CHIKV were observed.
Conclusions:
Ready-to-use lyophilized PCR detection kits represent fast, easy and useful systems for detection of tropical arboviruses, minimizing the time for reaction preparation and contamination problems, and allowing room temperature conditions for shipping and storage. "VIASURE *Zika, Dengue & Chikungunya* Real Time PCR Detection Kit" offers a reliable accuracy for ZIKV, DENV and CHIKV detection from a small amount of sample.

B-071

Comparison of two rapid antigen test kits of influenza virus and rRT-PCR test results

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Background: The method of rRT-PCR was used as a standard to evaluate the sensitivity of rapid antigen detection kits of two influenza virus in screening influenza A and B viruses, providing data support for selecting suitable methods in clinical laboratories. **Methods:** Totally 110 positive samples of influenza virus from fever clinic of the First Affiliated Hospital, College of Medicine, Zhejiang University were selected, including three common subtypes of sH3N2, H1N12009 and H7N9, as well as influenza B virus. Two kinds of rapid antigen detection kits of influenza virus (FDA, CE and CFDA approval) were tested. Meanwhile, the detection rate of these two kits in our hospital in 2016 was estimated. **Results:** The sensitivity of reagent A to influenza A viruses sH3N2, H1N12009, H7N9 and influenza B were 65.1%, 56.5%, 29.2% and 57.1%, while sensitivity of reagent B were 65.1%, 56.5%, 29.2% and 57.1%. The sensitivity of reagent A to influenza A and B virus in the ranges with threshold cycle (Ct) values of <25, 25-30 and> 30 were 82.9% (29/35), 51.7% (15/29), 15.4% (4/26) and 87.5% (7/8), 50% (4/8), 20% (1/5). Sensitivity of reagent B in each range were 62.9% (22/35), 24.1% (7/29), 0% (0/26) and 87.5% (7/8), 12.5% (1/8), 0% (0/5). In 2016, a total of 644 positive samples of influenza A virus were detected in our laboratory, accounting for 23.9% (154/644), 35.9% (231/644) and 40.2% (259/644), respectively, in the range with Ct values of <25, 25-30 and> 30. 106 positive samples of influenza B virus were detected, accounting for 35.8% (38/106), 18.9% (20/106) and 45.3% (48/106) for each ranges. The detection rate was estimated to be 45.3% if reagent A was used to screen for samples of influenza virus tested in 2016, while be 25.1% if reagent B was used. **Conclusion:** Sensitivity of rapid antigen test kit of influenza virus for different subtypes is different, higher sensitivity for higher viral loads while likely to be undetected for lower loads. Clinical laboratories should perform comprehensive performance verification before using these kits.

B-072

Contribution of Biomarkers in the Diagnosis of Sepsis in the Emergency Department

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Background: Sepsis is defined as a life-threatening organ disfuntion that is caused by a dysregulated host response to infection. Sepsis is a common condition handled in the Emergency Department (ED) and it causes millions of deaths globally each year. The research on accurate and timely diagnosis or exclude of suspected sepsis is vital to patient, which can reduce morbidity, reduces cost, and improves patient outcome. In this situation, the contribution of laboratory biomarkers is essential and so, in recent years, major efforts have been made to find biomarkers that allow early diagnosis of this disease. Procalcitonin (PCT) is the best investigated biomarker, and together with C-reactive protein (CRP), are the most frequently used biomarkers in clinical practice. Interleukin-6 (IL6) is widely investigated for its fast response to the infectious stimulus and Soluble CD14 subtype (Presepsin) is related to mediating the immune response in sepsis, but conclusive data for the application of these biomarkers are missing.The aim of this study was to investigate the diagnostic value of CRP, PCT, IL6 and Presepsin in the diagnosis of sepsis. **Methods:** 100 patients presenting at the ED with suspected sepsis were included. Blood samples were collected at first medical evaluation and CRP, PCT, IL6 and Presepsin were analyzed**.** CRP, PCT and IL6 measurements were determined in Cobas 8000 analyzer (Roche Diagnostics®) and Presepsin in Pathfast analyzer (Mitsubishi Chemical®). After diagnosis, the patients were divided is two groups: A (non-infectious etiology, localized infection or SIRS) and B (sepsis or septic shock). **Results:** The four biomarkers showed significant differences between groups (p=0.000 for PCT and IL6; p=0.034 for CRP; p=0.049 for Presepsin). The AUCs for the diagnosis of sepsis were 0.864 for PCT (p=0.000), 0.674 for CRP (p=0.044), 0.891 for IL6 (p=0.000) and 0.653 for Presepsin (p=0.047). The comparison between PCT and IL6, the two best biomarkers, did not reveal significant differences. Also no significant differences were found when comparing IL6 with the combination of CRP and PCT (AUC=0.822), the biomarkers currently used in our hospital. We developed a logistic regression model including CRP, PCT and IL6, and the AUC (0.929) was significantly higher compared to the use of biomarkers alone. The model AUC was also significantly superior to the combined use of CRP and PCT **Conclusion:** Presepsin provides a limited diagnostic value for sepsis, the worst of the four biomarkers evaluated. The diagnostic performance of IL6 is equivalent to the combined use of CRP and PCT, in both cases suitable for the identification of patients with sepsis. The addition of IL6 to the biomarkers already used, PCT and CRP, imply a significant improvement and represents the best diagnostic performance. Therefore, we recommend to include IL6 in the diagnostic algorithm of sepsis management in ED because it may assist clinicians in their decision making for early antimicrobial administration, enable risk stratification and expedite the execution of sepsis bundle.

B-073

assessement of the study of intestinal protozoan in the adult chronic diarrhea syndrome

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Background: Stool analysis is of special relevance in the study of adult chronic diarrhea syndrome and should include a microscopic study to assess the existence of leukocytes, blood or fats, in addition to the study of fecal calprotectin. With independence should be studied, infectious, bacterial and parasitic causes. The microscopic study of parasites in stools is a laborious technique that requires experience, and a high time of microscopic observation. Therefore, it is important to know the performance of this test in the context of chronic diarrhea syndrome in adults. The aim of this study is to evaluate the performance of the microscopic study of parasites in adult

patients clinically diagnosed with chronic diarrhea syndrome based on the calculation of post-test probability. **Methods:** A total of 302 adult patients (aged 16-96 years) with clinical presumption of chronic diarrhea syndrome were selected for the pilot study. The microscopic study of the faeces was carried out previous concentration of the sample by the Telemann method. The sensitivity and specificity values of the microscopic technique were calculated based on the data of the work published by *Stensvold et al. J Clin Microbiol 2012*. With these values, the probability coefficient was calculated which together with the pre-test probability (prevalence) were used to calculate the post-test probability. For the calculation of the negative post-test probability and its confidence interval, a methodology based on the Bayes theorem was used in Microsoft Excel based on the calculated prevalence of the disease in our pilot study and the results of the laboratory test. **Results:** In the microscopic study, 8 samples with parasites were detected, equivalent to a pre-test prevalence (prevalence) of 1.98%. All the parasites identified were protozoa: 3 *Endolimax nana* and 5 *Blastocystis hominis*. The negative post-test probability calculated wich Bayes' theorem was 1.7% (CI: 0.4 - 6.5%). **Conclusions:** - Based on the results, 5 out of 100 patients (based on the calculated confidence interval the number of patients would range between 1 and 20) studied for chronic diarrhea and parasitic etiology would not be diagnosed by microscopic techniques. - Therefore, based on the above, for the study of chronic diarrhea syndrome in adults, it would be appropriate to implement a contingent strategy with the use of more sensitive techniques such as molecular diagnosis, thus reducing the number of false negatives in the techniques conventional.

B-074

Mycobacterium tuberculosis: a validation of molecular test for detection of bacteria DNA and resistance to rifampicin

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Background: Tuberculosis (TB) has reached alarming proportions of 10.4 million incidence cases and 1.7 million deaths attributed to the disease as reported by the latest WHO global TB report 2017. In 2016, 66,796 new cases and 12,809 cases of tuberculosis retreatment were registered in Brazil. Globally, some 50 million individuals are already latently infected with multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains creating a remarkable resource for future cases of active TB. The four first-line drugs routinely used in anti-tuberculosis therapy are: isoniazid (INH), Rifampin (RIF), Ethambutol (EMB), Pyrazinamide (PZA). The WHO recognizes the urgent need for more accessible diagnostic tools that are rapid, accurate and associated with detection of resistance to drugs. The GeneXpert MTB/RIF assay (Cepheid's GeneXpert Dx System) was developed to improve TB and RIF resistance detection and to have minimal biological hazards. This system integrates and automates sample processing, nucleic acid amplification, detection of the target sequences using real-time PCR including the rifampin resistance-determining region (RRDR) of *rpoB* gene. **Objective**: Evaluate the performance of GeneXpert MTB/RIF System for molecular detection of *M. tuberculosis* and rpoB gene mutations to rifampicin in Brazilian infected patients in Institute Hermes Pardini (Vespasiano, Minas Gerais, Brazil). **Methods**: The GeneXpert MTB/RIF assay was performed following manufacturer's instructions. The respiratory specimens samples (sputum) were collected from patients with suspect of TB and they were sent to mycobacteriology diagnostics services on publics health centers (Belo Horizonte, Minas Gerais, Brazil). The results obtained were compared with ours for interlab evaluating. Three repetitions of a specimen were used to determine the intrassay precision and three repetitions of the same specimen in 3 days were used to determine the interassay precision of proposed method. **Results:** A total of 41 patients were processed by GeneXpert MTB/RIF kit. The results showed a concordance between the two centers of 48.7% (20/41) of negative specimens, and 46.3% (19/41) of susceptible specimens and 4.8% (2/41) resistant to RIF. The Kappa index was 0.952 (95% CI= 0.664 to 1.00), indicating almost perfect degree of agreement. A specimen was MTB detected low in public health center but in our laboratory the result obtained was negative MTB. The precision studies presented the same results in all conditions (intrassay and interassay), indicating good reproducibility. **Conclusions**: Our results demonstrate that the Xpert MTB/RIF assay can be used to diagnose of MTB and detection of resistance to RIF with basic laboratory infrastructure. The discordant result due probably the fact that in public center the detection of MTB was next to limit of detection and the result of culture was negative. Besides that the patient is HIV positive and has been treated for MTB for more than 10 years. Tuberculosis still remains a challenge to be overcome in Brazil, even though there are reductions in the incidence and mortality coefficients, the disease is still endemic in the country. The Xpert MTB/RIF assay is a WHO endorsed point-of-care molecular assay able to assess simultaneously diagnosis of MTB and RIF resistance, in approximately 2 hours so becomes a fast and accurate diagnosis.

B-075

Validation of a molecular test for detection and differentiation of Herpes Simplex Virus Type 1 (HSV-1) and 2 (HSV-2) in Institute Hermes Pardini, Brazil.

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Background: Herpes simplex virus 1 (HSV-1) and HSV-2 cause a spectrum of diseases that often present as lesions at oral or anogenital and central nervous system (CNS) sites. According to the World Health Organization (WHO) over half a billion people are estimated to have genital HSV infection globally, and HSV fuels the AIDS epidemic by increasing the risk of HIV acquisition and transmission. Early laboratory confirmation of these infections is performed by viral culture of the cerebrospinal fluid (CSF), or the detection of specific antibodies in serum. The sensitivity of viral culture ranges from 65 to 75%, with a recovery time varying from 3 to 10 days. Serological tests are faster and easy to carry out, but they exhibit cross-reactivity between HSV-1 and HSV-2. Currently, assays based on molecular techniques have been highlighted by clinical laboratories for being more sensitive and specific, and also reduce detection times. **Objective**: To validate a real-time PCR test for the differential detection of these viruses, and to compare it with a Nested-PCR. **Methods**: The samples were obtained from patients with presumptive diagnosis of HSV infection. The types of samples include were cerebrospinal fluid (CSF), whole blood, and genital mucosal samples. The DNA viral was extracted by a silica-based purification (in house method). As positive controls, commercial available viruses were used. For Nested-PCR, the amplicons were visualized in agarose gel electrophoresis. The amplification of real-time PCRs was performed in a 7500 Real-Time PCR System, using the TaqMan detection system with predetermined concentrations of primers and probes. **Results**: A total of 61 samples were examined by qPCR and Nested-PCR. Of these, 45 samples were found to be negative by both tests. The qPCR revealed 16 positive samples: 7 were positive for HSV1; 7 for HSV2 and 2 exhibited coinfection. Twelve samples were positive in Nested-PCR: 4 were positive for HSV1, 7 for HSV2 and 1 coinfection. The qPCR test had a limit of detection (LOD) of 20 copies/µL for HSV1 with a mean Ct value of 34, a standard deviation (SD) of 1.35 cycles, and a coefficient of variation (CV) of 3%. The LOD for HSV2 was 16 copies/µL, with mean Ct value of 29 (SD=1.2; CV=4%). The cross-reactivity test showed negative results when tested against CMV, VZV and EBV in 9 samples. The Kappa coefficient was 0,816 with 95% confidence intervals (CI) of 0,569 to 1.0, indicating nearly perfect agreement between the tests. **Conclusion**: The real-time PCR identified all positive samples detected in Nested-PCR, probably due to the higher sensitivity and not to lower specificity, since the test performance against others virus with potential of cross-reaction was excellent. Furthermore, the data indicated that the qPCR was well validated for the diagnosis of herpes and for the distinction HSV-1 and HSV-2 genome. Since this validation, real-time PCR can be used as part of the diagnostic algorithm of infections caused by these viruses. Accurate HSV detection and typing by molecular methods are considered the methods of choice this improves the diagnosis and guides the specific treatment.

B-076

Performance evaluation of the Beckman Coulter VERIS HBV assay in comparison to the ABBOTT RealTime HBV assay

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Background: The detection and quantification of HBV DNA are essential to diagnose chronic HBV infection, establish the prognosis of related liver disease, and monitor the virologic response to antiviral therapy. The aim of this study was to evaluate the analytical performance of the VERIS HBV assay in comparison to the ABBOTT RealTime HBV assay. **Methods:** Analytical performance of the VERIS HBV assay and method comparison with ABBOTT RealTime HBV assay was assessed according to the CLSI guidelines using 187 plasma samples including 20 drug-resistant HBV. **Results:** The between-day precision ranged from 4.15% for the mean 2.09 log IU/mL to 0.92% for the mean 4.68 log IU/mL. A linear relationship was found over 7 logs for HBV-DNA ($r^2 = 0.9994$; $P < 0.0001$). The lower limit of quantification was estimated at 8.76 IU/mL (95% CI: 7.32 to 12 IU/mL). For Bio-Rad controls, the total CVs were 3.62% (2.30 log U/mL), 2.27% (2.56 log U/mL) and 0.81% (4.38 log U/mL). The Passing-Bablock regression analysis showed good agreement between the VERIS HBV and the ABBOTT RealTime HBV assays in 187 samples ($y = -0.239713 + 0.971264x$), as well as in 20 drug-resistant HBV ($y = -0.541551 + 0.995370x$) samples. The mean differences between the VERIS

and ABBOTT assays were −0.3674 log IU/mL (95% CI, −0.4373 to -0.2974) in 187 samples and −0.44 log IU/mL (-1.40 to 0.51) in 20 drug-resistant HBV. **Conclusion:** The VERIS HBV assay is well-suited to monitoring HBV DNA levels in both chronic HBV and drug-resistant HBV, according to current clinical practice guidelines.

B-077

Prevalence of hepatitis C virus variants resistant to NS5A inhibitors in patients infected with HCV genotype 1b in Southern Taiwan.

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Background:
Hepatitis C virus (HCV) non-structural protein 5A (NS5A) inhibitors have been recently developed to inhibit NS5A activities and have been approved for the treatment of HCV infection. However, the drawback of these direct acting antivirals (DAAs) is the emergence of resistance mutations. The prevalence of such mutations conferring resistance to HCV-NS5A inhibitors before treatment has not been investigated in Chang Gung Memorial Hospital-Kaohsiung Medical Center. The aim of this study was to detect HCV variants resistant to HCV-NS5A inhibitors in hepatitis C patients infected with HCV genotype 1b before any treatment with NS5A inhibitors.
Materials and Methods:
Patients
The current study included 559 patients infected with HCV genotype 1b who were referred to gastroenterology department in our hospital between Dec. 2016 to May 2017.
NS5A amplification
Total RNA was extracted from 1 mL of plasma using Abbott mSample Preparation kit according to the manufacturer's recommendations. The extracted RNA was reverse transcribed using the PrimeScript 1st strand cDNA Synthesis system. First PCR of the HCV NS5A was amplified using the pair of primers as follows: sense 5′-AAGAGGCTCCACCAGTGGAT-3′ and antisense 5′-CGCCGGAGCGTACCTGTGCA-3′. One microliter from the first PCR reaction were used in the nested PCR with the pair of primers as follows: sense 5′-AATGAGGACTGCTCCACGCC-3′ and antisense 5′-GTGAAGAATTCGGGGGCCGG-3'. The nested PCR product obtained was 436 bp in size
NS5A direct sequencing and sequence analysis
The nested PCR products were purification using the DNA Clean & Concentrator according to the manufacturer's instructions. DNA sequencing was performed using the BigDye Terminator v 3.1 Cycle Sequencing Kit with a 3130 genetic analyzer. Nucleotide sequences were aligned with reference sequences AJ238799 for gentoype 1b. The threshold of nucleotide mixture detection during sequencing of sample is estimated to be around 20%.
Result:
The NS5A gene was successfully sequenced in 539 out of 559 (96.4%) samples that were amplified by PCR. Resistance mutation to NS5A region (substitutions of amino acid 28; 30; 31; 58 and 93) were observed in 204/539 (37.8%) sequences analyzed. Y93H (n=90; 16.7%) predominated over P58S (n=37; 6.86%), R30Q (n=21; 3.9%) , L28M (n=8; 1.48%) and L31I (n=4; 0.74%).
Conclusion:
Mutations conferring resistance to HCV NS5A inhibitors are frequent in treatment-naïve patients infected with HCV genotype 1b. Their influence in the context of DAA therapies has not been fully investigated and should be taken into consideration.

B-078

Determination of IgG antibodies to Measles, Mumps, Rubella and Varicella Zoster virus using a fully automated chemiluminescent multiplex analyser system

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Background
Routine detection of Measles, Mumps, Rubella and Varicella Zoster MMRV IgG is used to determine antibody status where infection history or previous immunisation is unknown.
Materials/Methods
This MMRV assay was developed using the Dynex Technologies Multiplier™ fully automated chemiluminescent multiplex analyser system and coated bead technology. Antigen coated beads representing each MMRV specificity were embedded into the base of the wells of the assay plate. Each assay well contains the 4 MMRV targets for the test sample IgG detection. The final chemiluminescent reaction is imaged with the on-board camera and results output as index values referenced against the assay specific calibrator. Precision was measured by assaying a range of 14 samples 3 times across an assay plate on three instruments over three days. A ROC analysis was run in order to set the cut-off for each of MMV and confirm it for Rubella where the cut-off was ultimately defined by the International reference RUBI194. Using the resulting cut-off values, the concordance was assessed on up to 929 samples collected for MMRV screening; results were compared to 510k cleared ELISA assays. Analyse-It® software was used for the ROC analysis and also to generate the 2x2 tables with a Wilson confidence interval set to 0.95%.
Results
Precision
The mean percentage coefficient of variation (%CV) for all four assays varied as follows:
Within run: 3.69-5.35%, between run: 4.06-5.66%, between day: 3.15-4.76% and between instrument: 1.37-3.19%.
ROC analysis
Area under the curve (AUC) and 95% CI results were: Measles 0.995 AUC (0.991-0.998 CI), Mumps 0.987 AUC (0.977-0.997 CI), Rubella 0.998 (0.997-0.999 CI) and VZV 0.999 (0.997-1.000 CI). Percent positive agreement (PPA) and percent negative agreement (PNA) with 95% confidence intervals (Cl) were calculated in two ways:
Equivocal samples scored as positive
PPA: Measles - 95.3% (93.5-96.6% CI), Mumps: 90.2 (87.8-92.2% CI), Rubella: 93.9 (91.9-95.4% CI), VZV: 98.1 (96.8-98.8% CI). PNA: Measles: 94.2 (90.7-97.0% CI), Mumps: 93.3 (88.5-96.2% CI), Rubella: 99.5% (97.4-99.9% CI), VZV: 97.5 (96.3-99.2% CI).
Equivocal samples scored as negative
PPA:Measles-93.3%(91.2-95.0%CI)Mumps:93.3(91.1-95.0%CI),Rubella:93.0(90.9-94.7% CI), VZV: 97.7 (96.3-98.5% CI). PNA: Measles: 95.4 (92.0-97.4% CI), Mumps: 94.6 (90.8-96.9% CI), Rubella: 100.0 (98.4-100.00% CI), VZV: 99.2 (95.6-99.9% CI).
Conclusion
This multiplexed fully automated assay gives reproducible semi-quantitative results for MMRV IgG. It is ideal for batch testing as it can handle up to ninety two test samples in a single plate to produce 368 results in <3 hours. When two plates are run together, 736 results are generated in 5 hours. *Under development. The performance characteristics of this device have not been established. Not available for sale, and its future availability cannot be guaranteed. The Multiplier is currently Research Use Only*

B-079

Comparison of different molecular assays to diagnose human respiratory viral infections

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Background: Respiratory infections are a major global health problem, mainly affecting young children and the elderly in low- and middle-income countries. The causative agents of this type of infections are viral or bacterial, being viruses more frequently involved. The management of the infections is crucial to prevent epidemics or pandemics, so accurate and specific diagnosis tools are required. The aim of this study is to compare two different Real-Time PCR assays with CLART®PneumoVir kit, which is the hospital routine diagnostic method. **Materials/methods:** 108 respiratory samples with a positive diagnosis by CLART®PneumoVir (Genomica) to some of the most common viruses that cause human respiratory infections were included in this prospective comparative study. The samples were collected at Hospital Clínico Universitario Lozano Blesa (Spain) during three years: 2014-2017, comprising different seasonal viruses' subtypes. All samples were analyzed by VIASURE Respiratory Panel (Certest Biotec), FTlyo Respiratory Pathogens 21 (Fast Track Diagnostics, FTD) and CLART®PneumoVir (Genomica). The two first assays are lyophilized ready-to-use Real-Time PCR products whereas the last one is based on reverse transcriptase amplification and visualization in low-density microarray. **Results:** The results are shown in the following table:

	VIASURE assay	Fast Track assay	Agreement with reference to CLART®PneumoVir (Genomica)	
	No. positive samples	No. positive samples	VIASURE vs Genomica	FTD vs Genomica
Influenza A/Influenza H1N1/ Influenza B	19	18	100%	94,7%
Coronavirus 229E/ Coronavirus NL63/ Coronavirus OC43	20	18	100%	90,0%
Parainfluenza 2/Parainfluenza 3/Parainfluenza 4	24	4	100%	16,7%
Parainfluenza 1/ Metapneumovirus/Bocavirus/ *Mycoplasma pneumoniae*	21	20	100%	95,2%
Respiratory syncytial virus A/B/Adenovirus	24	22	100%	91,7%

Total: 108 samples

Conclusions: VIASURE Respiratory panel exhibited as good clinical accurancy as CLART®PneumoVir with the additional advantage of being easier to perform and reducing turnaround time. FTD Respiratory Panel was not able to detect some positive samples with the inconvenience of requiring a three-fold increase in the starting amount of RNA template.

B-080

Urine Specimen Stability Comparison in Various Storage Conditions for Sediment Analyses on Atellica UAS 800 Analyzer

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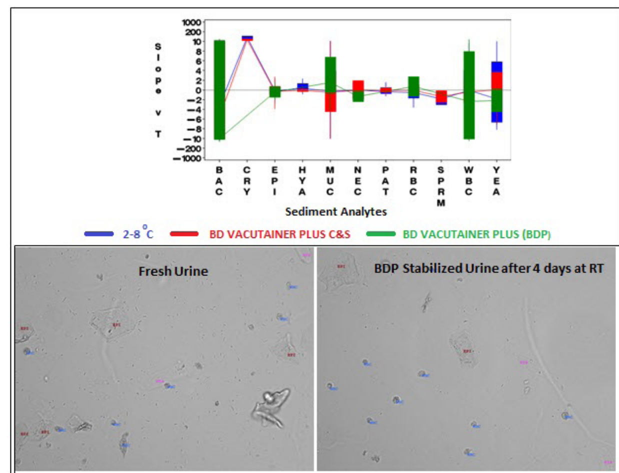
Background: Urinalysis is among most commonly used screening tests in clinical laboratories. Although automatic analyzers reduce analysis time, sample transportation time may lead to delayed preanalytical time. This logistical constrain challenges the laboratories to perform sediment urinalysis within recommended 4 hours. Earlier research reported contradictory stability results with limited number of analytes for up to 72 hours. In this study, the stabilities of 11 urine sediments particles were determined in five storage conditions for up to 96 hours. **Methods:** Urine specimens were received within 4 hours of collection. Several urine specimens were pooled to create 13 pools. Each pool was stored in five conditions: at room temperature, at 2-8°C without preservatives, and at room temperature in BD VACUTAINER PLUS, BD VACUTAINER PLUS C&S Preservative and BORITEX (Aldwin Scientific) urinalysis preservative tubes. The sample-pools (3 aliquots) were analyzed for 4 days in 3 replicates/run on Atellica UAS 800 Analyzer* (Siemens Healthineers). The stability of each analyte was estimated by determining the slope of a regression fit to the recovered sediment particle results as a function of time. The slope of the relationship was considered statistically non-significant if $p > 0.05$. **Results:** The results indicate room temperature storage caused significant bacterial growth. The results also indicate that the slopes for WBC, RBC, EPI, NEC, BAC, HYA, PAT, YEA, MUC are non-significant at 2-8°C and at room temperature with preservatives for up to 4 days. The results further indicate the CRY slope is significantly positive at 2-8°C; SPRM slope is significantly negative. **Conclusion:** The results suggest that the several urine sediment analytes are stable at 2-8°C without preservative and at room temperature with the preservatives for up to 4 days. CRY and SPRM analytes demonstrated significant instability under all storage conditions evaluated. * Not available for sale in the U.S. Product availability varies by country.



B-081

Prevention of Highly Pathogenic Avian Influenza Virus from Poultry/ Humans and Prediction of Its Outbreak by Satellite

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Background: One third of world population were killed during the 1918 influenza pandemic with half a million deaths only in the United States, as summarized by AACC in 2016. The aim of the present study was to propose the simple and cheap measures for protection of poultry from avian influenza virus (AIV) with cross-species transmission to humans as H5N1, H2N2, H9N2, H7N7. **Methods:** Control and Experiment were compared to see the viral inactivity under measures of chemical, biological, and physical methods in the laboratory in vitro tests of H5N6 and H1N1. **Results:** The present methods showed that the highly pathogenic influenza (HPAI) A virus subtypes have lost the viral activity after treatments of chemical, biological, and physical measures to inactivate the virus. Furthermore, the predictions of place and time of HPAI outbreaks were determined by data of remote sensing satellite from NASA prior to their occurrences in all over the world, including regions without HPAI outbreaks. The initiative results were disclosed on the basis of the incredible linear relationship ($R^2 = 0.9967$) between the year of the AIV outbreaks and the year of minimal average daily sunspot area during 1878 to 2016. **Conclusion:** The source of AIV were penguins in Antarctica and guillemot in Arctica. Migratory birds and humpback whales transmitted low pathogenic avian influenza (LPAI) to the AIV sink of Continents with rice, wheat, maize, waters, and mudfishes persisted and mutated as HPAI under low UV-B exposure, temperature, salinity, relative humidity, and desert dust particles to infect the domestic poultry and humans with HPAI. The present simple measures may save the Earth from HPAI in domestic poultry and humans.

B-082

New sampling strategies to detect environme ntal microbial contamination and to verify disinfection and sterilization procedures

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Background: Hospital-acquired infection are often connected to contamination of inanimate surfaces near the patients. Up to day, there is not standardize and efficient methods to evaluate the microbial contamination and consequently assess the efficacy of the cleaning procedures. The sampling of the surfaces can be performed using contact plates or swabs. Contact plates are used for sampling of flat

surfaces. Swabs are used for sampling of articulated surface. Aim of the study was to investigate a new device for surface monitoring, the FLOQSwab in combination to SRK solution (Hygiene Monitoring System HMS, Copan Italia) to evaluate the efficacy of the sanitization method used to clean surfaces in Hospital wards **Methods:** The following Hospital wards were considered for the monitoring: Dialysis Center (n=5 sampling points); Gynecology Surgery Room (n=14 sampling points) and Orthopedic Radiology (n=5 sampling points). Cleaning procedure: identified sampling points were cleaned using a disinfection system (HyperDRYMist® technology). Sampling was performed in parallel before and after the cleaning procedure with a new device and the traditional swab. To standardize the area to be sampled, a square cardboard frame 10 x 10 cm (COPAN Italia) was used to define the area for testing. The flocked swab was transferred in its transport medium tube (1mL of SRK solution) and the traditional swab in 1ml of saline solution. The whole 1 ml was used to inoculate Tryptic Soy Agar plate at 35°C up to 3 days. The bacteria identification was performed by mass-spectrometry **Results:**The efficacy of the sanitization procedure was evaluated on the difference in colonies count detected on the surface before and after sanitization. In all wards considered, the use of HMD has allowed to identify more bacteria species then the traditional swab. In all the sampling points, HMD was able to detect on the different surfaces the "thrue microbial load", the rayon swab reported an underestimation of the microbial load in all analyzed sampling sites.**Conclusion:**Use of Nylon Flocked swabs as improved swab sampling device and SRK solution as preservation medium allowed to adequately assess the microbial contamination on the surfaces sampled and thus properly evaluate the effectiveness of disinfection system used.

B-083

In vitro starvation model for Assessing Phenotypic Drug Tolerance on Mycobacterium Tuberculosis Lineages in Ethiopia

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Abstract
Background: Mycobacterium tuberculosis persist in the human host for decades& reactivation can occur at any point. Becomes dormant and phenotypically drug tolerant when exposed adverse conditions. Understanding of the signals and processes which allow the bacteria to achieve this feat could potentially be used as a baseline to design new types of drugs or modify old drug regimens for improved cure and avert development of drug resistance. **Objective:** To use *in vitro* starvation model in assessing if nutrient deprivation affects phenotypic drug tolerance in *Mycobacterium tuberculosis* lineages circulating in Ethiopia. **Methods:** Three MTB lineages and one standard susceptible reference strain (H37Rv) were tested by different test methods at different time point from March to September 2017. All lineages tested to be sensitive to first line anti Tb drugs. Log phase (highest colony count on week 3-4) culture from Lowenstein Jenson medium sub cultured to Middle-brook7H9 with 10% Oleic Acid Albumin Dextrose Catalase as a normal, Phosphate Buffer Solution (PBS) (PH 7.2) and Sterile Distilled water (SDW) as starvation media were used. Each week we performed culture growth reading, Acid Fast Stain (AFS) by Zeihel Nelson (ZN), Lipid Bodies (LB)by Sudan black stain and viability by Fluorecin DiAcitate (FDA) staining. On week 0, 3 and 6 drug susceptibility test was done by colorimetric MTT assay. Graph pad prism 6 and SPSS V20 used for data analysis. **Results**: A total of 576experiments were performed using4 strains of *Mycobacterium Tuberculosis*subcultured on SDW, PBS and 7H9 and. Of these, 324 microscopic tests using (108(ZN) acid fastness, 108(FDA) viability, and 108(Sudan black stain) lipid bodies), 108 culture growth reading done. After week 6acid fastness, viability and culture growth decreased. 144 phenotypic DST done using MTT assay. A higher inhibitory drug concentration was required at the 6th week compared to the baseline and C50 (RMP=0.5; INH=0.1; STM=2.0 and for EMB=4.0), yet the proportion of lipid body containing bacilli increased continuously in all lineages. **Conclusion**: Our study showed that the mycobacteria lineages behaved similarly in all media systems and reached stationary phase at similar time. The increased drug concentration observed at the 6th week coincided with the decline in viable bacilli in all media systems, thus attributing this phenomena to lipid body accumulation alone was difficult.
Keywords: M. tuberculosis, LB%, Drug Tolerance, and MTT Assay.
1

B-084

Performance Evaluation of the VITROS® Immunodiagnostic Products B·R·A·H·M·S PCT Assay on the VITROS 3600 Immunodiagnostic Systems

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Background: We have evaluated the performance of VITROS® Immunodiagnostic Products B·R·A·H·M·S PCT (Procalcitonin) assay (in development), which consists of VITROS® B·R·A·H·M·S PCT Reagent Pack and the VITROS® B·R·A·H·M·S PCT Calibrators on the VITROS® 3600 Immunodiagnostic Systems using Intellicheck® Technology. **Methods:** The VITROS® B·R·A·H·M·S PCT assay is a two-step dual monoclonal immunometric assay that uses anti-PCT antibody immobilized on the well surface to capture PCT in the patient sample. Unbound PCT in the sample is removed by washing and the detector antibody (anti-PCT Mab) horseradish peroxidase (HRP)-labelled conjugate is added. Unbound HRP conjugate is removed by a second wash and the bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrate (a luminol derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the System. The amount of HRP conjugate bound is directly proportional to the concentration of PCT present in the sample. The time to first result in the system is 24 minutes. **Results:** The assay is calibrated against the B·R·A·H·M·S PCT™ sensitive KRYPTOR™. Limit of quantitation was determined to be 0.006 ng/mL. Linear regression analysis showed linearity across the range of 0.011 to 95.58 ng/mL. Precision study over 22 days with five precision pools showed excellent precision with sample concentrations of 0.040 ng/mL, 0.429 ng/mL, 1.69 ng/mL, 8.63 ng/mL, and 46.38 ng/mL resulting in within-laboratory percent coefficient of variation (%CV) of 7.5%, 3.0%, 3.0%, 5.0%, and 3.4% respectively. Patient samples showed acceptable results up to 20-fold dilution. No evidence of high dose hook was observed up to 5,000 ng/mL. The accuracy of the VITROS® B·R·A·H·M·S PCT assay was evaluated with 210 patient specimens (range: 0.11 to 93.38 ng/mL) against the B·R·A·H·M·S PCT sensitive KRYPTOR. The following regression statistics using Passing and Bablock was obtained: VITROS PCT = 0.98* B·R·A·H·M·S PCT sensitive KRYPTOR- 0.04; Pearson Correlation Coefficient (r) = 0.98. No significant interference or cross-reactivity were observed with biotin (3,500 ng/mL), conjugated bilirubin (32.3 mg/dL), unconjugated bilirubin (47.6 mg/dL), hemoglobin (500 mg/dL), heparin (8000 IU/L), total protein (1.65 g/dL), triglycerides (17.76 mg/mL), HAMA (>160 IU/mL), and RF (282 IU/mL). The samples can be stored up to 24 hours at room temperature, 48 hours refrigerated and up to three freeze-thaw cycles. Serum, EDTA and lithium heparin matrices showed acceptable results. The reference range using negative samples based on central 95th percentile was 0.004 ng/mL to 0.037 ng/mL. The VITROS B·R·A·H·M·S PCT assay showed excellent negative and positive percent agreements compared at the B·R·A·H·M·S PCT sensitive KRYPTOR at medical decision cutoff of 0.10 ng/mL, 0.25 ng/mL, 0.50 ng/mL and 2.0 ng/mL.**Conclusion:** In summary, the VITROS® B·R·A·H·M·S PCT assay demonstrates reliable and acceptable performance on the VITROS 3600 Immunodiagnostic Systems.

B-085

Active Surveillance Cultures: Frequency of Microorganisms and Phenotypic Resistance Profile from Public Hospitals of Sao Paulo City, Brazil

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Background: Surveillance cultures are routinely used by public health authorities to screen for multidrug resistant bacteria. In the past few decades, the widespread use of broad spectrum antibiotics has provided the acquisition of resistance genes that in general are carried by plasmids, which contribute to rapid spread of resistance genes within the bacterial population. The purpose of this study was to determine the prevalence of multidrug-resistant (MDR) organisms isolated from rectal swab screening in several Brazilian public hospitals represented by the north, south, east and west regions of the São Paulo city. **Methods:** Surveillance samples were analyzed from 1st January to 30st December 2017. All the rectal swab samples were collected from patients admitted from high-risk settings or transferred from areas with high rates of MDR organisms. All clinical specimens were inoculated onto a selective media (ChromID media, bioMerieux) and incu-

bated at 35±2°C for 18-24 h. The screening test was performed to Gram-negative bacilli (GNB) using the antibiotics ertapenem, imipenem and meropenem and for Gram-positive Cocci (GPC) screening was tested oxacillin (methicillin) and vancomycin using disc diffusion method. Bacteria identification was performed by MALDI-TOF mass spectrometry (Vitek-MS, bioMerieux) and, as required, the minimal inhibitory concentrations (MIC) of antibiotics were determined using the Vitek 2 System (bioMerieux). **Results:** A total of 22.641 rectal swab samples were processed. 3.084/22.641 (13.62%) showed positive results for the presence of microorganisms. Out of which, 2.344 (76%) were Gram-negative Bacilli (GNB) and 740 (24%) were Gram-positive Cocci (GPC). The majority of the isolated carbapenems resistance was *Klebsiella* spp. [1.872/3.084 (60.7%)], which the *Klebsiella pneumoniae* was the predominant species, followed by *Acinetobacter baumannii* [215/3.084 (7%)], *Pseudomonas aeruginosa* [125/3.084 (4%)], *Enterobacter* spp. [102/3.084 (0.97%)] and others BGN [30/3.084 (0.1%)]. Between the GPC the resistance to vancomycin was observed mainly in 16.8% *Enterococcus faecalis* (519/3.084) and 7.1% *Enterecoccus faecium* (220/3084). **Conclusion:** The findings in this study corroborates with other Brazilian studies, which *K. pneumoniae* carbapenem resistant was the most frequent organism recovered from rectal swab samples followed by *Enterococcus* spp. vancomycin resistant. Early colonization detection by screening assays should be used to minimize the chance of transmitting MDR organisms from colonized to non-colonized patients, reinforce the continued need for infection control hospital surveillance system.

B-086

Performance of Bio-Rad Laboratories HIV Quality Controls on the VITROSâ Immunodiagnostic Products HIV Combo assay.

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Background: Even with advanced blood screening and therapeutic options, HIV contamination or infection remains a serious threat to the world's blood supply and a major health issue for at risk populations. Constant vigilance, in the form of diligent screening of donated blood and accurate diagnosis in potentially infected people, is necessary to prevent a resurgence of this disease. Organizations such as the US Centers for Disease Control (CDC) and the World Health Organization (WHO) have defined very rigorous regimes for HIV testing, which include screening and confirmatory assays. An integral part of this testing regime is the use of third party quality controls, which confirm the reliability of these important tests and allow for a large measure of confidence in the reported results. **Objective:** The performance of five Bio-Rad HIV Quality Controls on the recently introduced Ortho Clinical Diagnostics VITROS HIV Combo 4th generation assay was examined. The controls (and analytes) were as follows: VIROTROL I (anti-HIV-1), VIROTROL HIV-2 (anti-HIV-2), VIROTROL HIV-1 Ag (HIV-1 Ag), VIROTROL HIV-1 gO (anti-HIV-1 gO) and VIROCLEAR (negative control). VIROTROL HIV-1 gO is not intended for use with blood screening assays. The VITROS™ HIV Combo Assay is for the simultaneous qualitative detection of antibodies to HIV-1 (including group M and O) HIV-2 as well as HIV p24 antigen in human serum and plasma using a chemiluminescent immunoassay (ChLIA) methodology. The VITROS HIV Combo assay is intended for diagnostic purposes only. The effectiveness of the VITROS HIV Combo assay for blood and/or plasma screening has not been established in the United States. **Method:** The evaluation of the Bio-Rad Laboratories HIV Quality Controls was performed using three different lots of the VITROS™ HIV Combo assay and utilized the VITROS™ ECi/ECiQ, 3600 or 5600 immunodiagnostic systems. The thirty week study was performed at three sites resulting in a total of 214 data points for each control (and analyte). Unopened samples of each Bio-Rad control were tested either daily or at approximately four week intervals, depending on the test site. **Results:** Test results for the controls were 100% in agreement with the expected "reactive" or "non-reactive" result, depending on the intended use of the quality control. **Conclusion:** Bio-Rad VIROTROL and VIROCLEAR controls are optimally suited for use as third party quality control materials on the Ortho Clinical Diagnostics VITROS HIV Combo assay, ensuring confidence in overall test performance and reported patient diagnostic test results.

B-087

Comparison of Abbott Architect Syphilis TP test and Bio-Rad Syphilis IgG test on BioPlex 2200.

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Background: Implementation of Beckman Automation Line (Power-Express) with directly attached Abbott Architect instruments (one of the first in the USA) prompted us to compare 2 methods of reverse algorithm syphilis testing between Bio-Rad Syphilis IgG kit on BioPlex 2200 (used in our lab for last few years) with Abbott Syphilis TP testing on Architect (qualitative detection of antibodies IgG and IgM directed against Treponema Palidum). **Methods:** Consecutive 1007 patients samples were tested in both systems. According to our policies, all positive or equivocal samples on BioPlex instrument were followed with RPR and TPPA testing. Additionally all positive samples on Architect had PRP and TPPA performed regardless of BioPlex results. **Results:** From the pool of 1007 patients, 857 had negative and 137 had positive results on both instruments(Cohen's kappa agreement 94,8 %).From 5 equivocal samples on BioPlex 3 were non-reactive and 2 reactive on Architect, RPR and PTTA.There were 5 reactive samples on BioPlex which were non- reactive on Architect, RPR and TPPA. There were 2 reactive samples on Architect which were non-reactive on BioPlex, RPR and TPPA.One sample reactive on Architect was non-reactive on BioPlex but reactive for RPR and TPPA. **Conclusion:** Our study confirms good agreement between these 2 methods of reverse algorithm syphilis testing.Minimal differences between these methods could be partially explain by design of the tests with equivocal zone on BioPlex 2200 and additional detection of IgM antibodies in Architect test.

B-088

Integrating exosomal microRNA and electronic health data to promote tuberculosis diagnosis

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Background: Tuberculosis (TB) is difficult to diagnose from complex clinical conditions. Diagnostic information from electronic health records (EHR) remains insufficient. Currently, exosomal miRNAs are emerging as biomarkers for diseases. We aim to investigate the potential of exosomal miRNAs and EHR in TB clinical diagnosis. **Methods:** 388 individuals were interrogated with a prospective multi-stage approach. Exosomal miRNA expressions were profiled with microarray followed by qRT-PCR. EHR and follow-up information of patients were collected accordingly. In discovery phase, differentially expressed miRNAs (DEM) were narrowed down and further selected. In selection and testing phases, models with 'EHR + miRNA' and 'EHR only' were established using support vector machine. We relieved the overfitting problem with unsupervised approach, model interpretation and testing phase. We in silico predicted the targeted genes of DEM, networks of DEM with related GO or KEGG pathways. **Results:** 351 individuals were finally enrolled. Six DEM (20a, 20b, 26a, 106a, 191, and 486) were over-expressed in pulmonary tuberculosis (PTB) and tuberculous meningitis (TBM) patients as compared with their controls. 'EHR + miRNA' model showed a better diagnostic efficacy for TBM than 'EHR only' model (AUC: 0.87 vs 0.70, sensitivity: 0.83 vs 0.71, specificity: both 1). Modelling with or without miRNAs both achieved satisfactory performance for PTB. DEM presented a decreased trend after 2-month intensive therapy (adjusted-p = 4.80 ×10-5). DEM were predicted to involve in immunologically regulation and neurotrophin receptor signaling. **Conclusion:** Our present study identified 6 exosomal miRNAs as promising noninvasive biomarkers for PTB and TBM patients. Combination of exosomal miRNAs and EHR through machine learning algorithm could serve as a feasible approach in promoting TBM differential diagnosis, and further prospective validation is required before its clinical utility.

B-089

Novel rapid quantification method of bacteria in a septic blood sample can produce an effective biomarker for monitoring patient care

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Background: Severe systemic infections, such as sepsis, are the primary cause of morbidity and mortality in hospitalized patients. Current biomarkers in sepsis do not always reflect the severity of sepsis at a particular point in time. Acquiring the earliest possible identification of pathogenic microorganisms is critical for selecting the appropriate antimicrobial therapy and obtaining a favorable outcome in infected patients. Here we developed a novel rapid identification and quantification method of unknown pathogenic bacteria in a clinical sample, and estimated the usability of blood bacterial concentration as a novel biomarker in sepsis. **Methods:** We have already reported the development of a rapid diagnostic method, called the Tm mapping method, which requires neither microbial cultures nor DNA sequencing to identify the causative pathogenic bacteria. This method is based on real-time PCR with seven primer sets, and the algorithm generates a unique "finger-print" of the bacterial species from the data of the melting temperature (*Tm*) of each PCR amplicon. This "finger-print" is compared with those of more than 150 bacterial species in the database. The software and database is accessible by Internet, and the output is the list of the bacterial species in the order of the matching score, called Difference Value. As a result, we can get an identification result of pathogenic bacteria around four hours after whole blood collection. In this research, we tried to improve the Tm mapping method to not only identify but also quantify bacteria in a sample. **Results:** We identified and quantified pathogenic bacteria in 26 septic blood samples, and the blood bacterial concentrations were correlated with the severity of sepsis (qSOFA, septic shock, Pitt Bacteremia Score). We subsequently examined the time-dependent changes (pretreatment, and 24 to 72 hours after antibiotic treatments) of blood bacterial concentration, and found that the time-dependent changes of blood bacterial concentration were dramatically decreased compared with the change of Body temperature (BT), White blood cells (WBC), C-reactive protein (CRP), Procalcitonin (PCT), Presepsin (P-SEP) and Interleukin-6 (IL-6). **Conclusion:** We developed a novel rapid identification and quantification method of unknown pathogenic bacteria in a whole blood sample, and found that the blood bacterial concentration would be useful as a novel biomarker not only to estimate the severity of sepsis but to monitor the therapeutic effect.

B-090

One-Step Real-Time PCR assay using a novel primers-probe set for universal detection of Dengue virus

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Background: Dengue virus (DENV) infection is the most important arthropod-borne viral infection of humans and the incidence of dengue has grown dramatically. According to WHO is estimated there are up to 390 million DENV infections annually, with more than 500,000 hospitalizations and 25,000 deaths. The Dengue virus group consists of four serotypes (DENV-1, DENV-2, DENV-3 and DENV-4) that manifest a diverse range of symptoms. Given that dengue virus infection elicits such a broad range of clinical symptoms, early and accurate laboratory diagnosis is essential for appropriate patient management. Molecular methods such as RT-qPCR have become a primary tool to detect virus in the early course of illness. In addition, molecular testing allows provide same- or next-day diagnosis of DENV during the acute phase of disease, thus permitting the monitoring of outbreaks and the implementation of control measures. **Objective**: To describe the validation of a one-step real-time PCR (RT-qPCR) using a novel universal sets of primers and hybridization probes for detection of dengue virus serotypes 1-4 in serum samples. **Methods**: The primers and probe were designed using the Primer Explorer V4 software. To assure the specificity of the primers, the 3' untranslated region of all complete genome sequences of dengue virus was selected and downloaded from GenBank, and aligned with multiple sequence alignment tools to identify the conserved region. Performance of one-step real-time PCR was evaluated using commercial controls. The ability of the assay to detect DENV in clinical samples was tested in 14 serum samples obtained from patients who had presented with dengue-compatible symptomatology and were confirmed to be DENV positive by standard laboratory diagnosis (Nested PCR). The amplification efficiencies and detection limits of this assay were determined. **Results**: A BLAST search against all available sequence databases at NCBI and an in silico PCR did not identify any additional homologous sequences, suggesting adequate performance and high specificity of the designed primers. The detection limits of the studied assay were 30 copies/reaction for DENV-1 and DENV-3 and 15 and 60 copies/reaction for DENV-2 and DENV-4, respectively. The regression equations obtained show good amplification conditions with positive correlation between the variables, with a coefficient of determination (r^2) varying from 0.86 to 0.98. The results obtained with clinical samples showed that 12 samples were positive for DENV and that the assay did not cross-react with other human pathogenic flaviviruses. **Conclusion**: The results suggest that this primer-probe combination could be the basis for development of new real-time PCR assay for laboratory diagnosis of dengue infection. In this method the reverse transcription and PCR processes are conducted consecutively on a real time PCR system. The rapid detection of the DENV by one-step real-time RT-qPCR has become a trend in diagnostic medicine. The proposed assay is efficient, sensitive, specific and less labor-intensive compared to the nested PCR. Advances in molecular methods have improved the sensitivity and specificity of diagnosis of dengue virus infection. It is expected that the application of these assays will contribute significantly to the clinical treatment, etiologic investigation, and control of this infection

B-091

Elevation of D-dimer is linked to disease severity and predicts fatal outcomes in H7N9 infection

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Background: To assess whether an increased D-dimer levels was related to worse global, renal, heart, and respiratory outcomes in critically ill patients with H7N9 infection and whether D-dimer could serve as a biomarker of severity. **Methods:** D-dimer levels in Plasma were serially measured on day 1, day 7, day 14 and day 21 of admission for 130 H7N9 patients (45 lethal and 85 non-lethal cases). 79 H1N1 patients and 71 healthy volunteers were selected as controls. To assess clinical illness severity, both APACHEII scores and the Pneumonia Severity Index (Pneumonia Severity Index class) were calculated. **Results:** Plasma D-dimer level in H7N9 patients was significantly higher than those in H1N1 patients and normal controls (P <0.001).The plasma D-dimer level in death group was significantly higher than that in survival group (P <0.001). Plasma D-dimer level in survival group was significantly lower than that on day 1 (P <0.001). Plasma D-dimer level in death group increased sharply from the day 7 to the day 14 after admission, and decreased significantly from the day 14 to day 21, with statistically significance (P <0.05). Plasma D-dimer levelswere positively correlated with hypersensitive C-reactive protein (HsCRP) and procalcitonin (PCT), liver indicators(ALT and AST)and cardiac indicators (CK, CKMB, LDH), as well as severity indicators PSI and APACHEII scores (r = 0.408 and 0.325, P <0.001). The area under the ROC curve for prediction of patient death at a plasma D-dimer level of 3943 ug/L FEU was 0.811, with a sensitivity of 81.6% and a specificity of 73.8%, better than HsCRP and PCT. The survival rate of the groupof patients with D-dimer> 3943 ug/L FEU was significantly lower than that of patients with D-dimer≤3943 ug / L FEU (P = 0.024). **Conclusion:** Plasma D-dimer levels have certain correlation with the severity and prognosis of H7N9 avian influenza. The higher the plasma D-dimer level, the lower the survival rate of H7N9 patients. Monitoring D-dimer levels can help physicians to determine the severity and prognosis of H7N9 avian influenza. **Financial support:** This work was supported by the China NationalMega-Projects for Infectious Diseases (grant number 2017ZX10103008); and the National Natural Science Foundationof China (grant numbers 81672014 and 81702079).

B-092

Neutropenia has a limited effect on plasma calprotectin levels

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Background: Antibiotics resistance is a growing problem worldwide and there is a need for better markers for bacterial infections to be able to distinguish between bacterial and viral infections. Plasma calprotectin may be an interesting early marker for bacterial infections. Calprotectin is manly expressed in neutrophil granulocytes, but is also found in macrophages and monocytes. A potential problem could be that calprotectin cannot be used in patients with low neutrophil counts or in case when neutrophils are attracted to the site of inflammation and are not present in the circulation. The aim of this study was to evaluate the association between neutrophil counts and plasma calprotectin levels.**Methods:** The study was performed

at Uppsala University Hospital. Plasma calprotectin was measured in Li-heparin plasma on a Mindray™ BS-380 (Mindray Medical International, Shenzhen, China) with reagents from Gentian (Moss, Norway). The instrument settings for the method were: sample volume = 3 µL, R1 volume = 200 µL and the R2 volume = 30 µL. The wavelength was 605 nm and the total assay time was approximately 10 min. The calprotectin values in the study cohort varied between 0.09 and 33.1 mg calprotectin/L. The patients had neutrophil counts in the range <0.1-16.1 x 10^9/L. The calprotectin levels in the samples (n=56) were correlated with the neutrophil counts. **Results:** There was a very weak association between the neutrophil counts and calprotectin levels ($y = 0.28x + 1.25$, $R^2 = 0.037$). Even patients with neutrophil values <0.1 x 10^9/L had detectable calprotectin levels. **Conclusion:** In our study neutropenia had a limited effect on calprotectin levels. The results indicate that calprotectin could also be used in patients with low neutrophil values. Further studies are needed to study the use of plasma calprotectin in different patient populations.

B-093

Turnaround time of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

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Background: Xpert MTB/RIF assay (Xpert) has the potential to rapidly diagnose pulmonary tuberculosis. The purpose of this study was to evaluate turnaround time (TAT) of Xpert during routine clinical use in an intermediate burden setting. **Methods:** Between July 2014 and December 2016, a total of 2,952 consecutive respiratory specimens were simultaneously tested by Xpert, mycobacterial culture, and smear microscopy. **Results:** Compared with smear microscopy, the median TAT of Xpert was significantly shorter (median [interquartile range, IQR] 3.1 [2.3-5.6] hr versus 19.1 [6.8-21.9] hr, $P < 0.0001$). When the time limits were stratified within 3, 6, 12, and 24 hours, the cumulative TAT compliance rates of Xpert were significantly higher compared with smear microscopy (within 3 hours, 49.1% [1,450/2,952] versus 0.4% [13/2,952], $P < 0.0001$; within 6 hours, 76.8% [2,267/2,952] versus 16.7% [492/2,952] $P < 0.0001$; within 12 hours, 80.5% [2,375/2,952] versus 41.4% [1,222/2,952] $P < 0.0001$; within 24 hours, 96.3% [2,842/2,952] versus 88.7% [2,619/2,952], $P < 0.05$, respectively). Bland-Altman analysis for TAT differences of individual specimens between Xpert and smear microscopy showed that Xpert had faster TATs than smear microscopy in 94.5% (2,791/2,952) of specimens. Moreover, the addition of one Xpert module significantly shortened the mean TAT from 3.7 hours (2.5-6.4 by 1 module) to 2.6 hours (2.1-4.6 by 2 modules) ($P < 0.0001$). **Conclusion:** The median TATs of Xpert were remarkably shorter than those of smear microscopy. Moreover, Xpert displayed a higher TAT compliance rate within 24 hours than smear microscopy. Collectively, our findings suggest that the ability for Xpert to rapidly report results may have a clinically profound impact on tuberculosis treatment initiation in an intermediate tuberculosis-burden setting.

B-094

Performance Evaluation of the Atellica IM HBsAgII (Qualitative), Atellica IM HIV Ag/Ab Combo (CHIV) §, and Atellica IM aHCV§ Assays at Two Hospital Sites

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Background: We evaluated recently introduced automated immunoassay analyzer Atellica IM 1600 (Siemens Healthineers, NY, USA) for detecting serologic Hepatitis C Virus (HCV), Human Immunodeficiency Virus (HIV), and Hepatitis B surface Antigen (HBsAg) markers by comparison with the results obtained from ARCHITECT i4000SR (Abbott Diagnostics, Abbott Park, IL, USA). **Methods:** For each HCV, HIV, and HBsAg study, over 1000 hospital routine samples prospectively assayed on Abbott ARCHITECT (both negative and positive for HCV, HBsAg and HIV-1 group M and HIV-2) were then tested on the Atellica IM 1600 Analyzer. The diagnosis of positive results was based on relevant marker profile and clinical and serological data available. For all the discordant results with ARCHITECT, samples were repeated on both methods. If discordant results remained, when possible, further testing was performed: nucleic acid testing and Siemens Healthineers R&D for HCV, confirmatory tests for HIV, and neutralization testing for HBV. Precision for the Atellica IM Analyzer assays was performed according to CLSI EP15-A3: Samples comprised Atellica IM HCV, CHIV, and HBsAg positive QC, BIORAD QC, and a plasma pool at a concentration close to the cutoff – one run per day, five replicates per run, for five days, for a total of 25 replicates per sample. Agreement was calculated vs. respective ARCHITECT assays. **Results:** Precision studies agreed with the manufacturer's claims. Preliminary concordance for HCV was 98.5%; for HBsAg was 99.8%; and HIV 99.9%. Discordant samples are under investigation and final sensitivity and specificity will be calculated. **Conclusions:** The Atellica IM HCV, CHIV, and HBsAgII assays demonstrated acceptable precision on the Atellica IM Analyzer, and good agreement with the Abbott Architect HCV, HIV Ag/Ab Combo, and HBsAg assays even though discordant samples require further investigation.

Precision assays according to CLSI EP15-A3

Atellica IM Analyzer Assay	Mean index	Within run %CV(SD)	Within lab (total) %CV(SD)
HCV	0.10	4.5(0.0)	4.9(0.01)
	1.25	1.9(0.02)	3.7(0.05)
	3.28	2.6(0.08)	3.1(0.10)
	4.34	2.7(0.12)	3.6(0.16)
CHIV	0.16	8.3(0.01)	8.3(0.01)
	0.96	1.4(0.01)	3.3(0.03)
	3.55	1.7(0.06)	2.7(0.10)
	4.75	1.9(0.09)	3.2(0.15)
	4.89	1.3(0.06)	3.0(0.15)
	6.09	2.1(0.13)	2.6(0.16)
HBsAg	0.12	13.9(0.02)	14.0(0.02)
	0.80	5.1(0.04)	5.1(0.04)
	5.80	2.7(0.16)	2.7(0.16)
	5.83, 9.93	1.8(0.10), 1.9(0.18)	1.9(0.11) 2.5(0.24)

B-095

Use of BACTEC MGIT 960 System to growth for Mycobacteria from clinical specimens in association of Public Hospitals Northern Anatolian Region of Istanbul

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Background: Tuberculosis continues to be a major health problem worldwide and also our country. Rapid and accurate diagnosis is key to controlling the disease. The traditional tests for TB produce results that are either in accurate or take too long to be definitive. Recent advances in new techniques have shortened the time needed to diagnose tuberculosis, leading to improved case detection and management; however, culture is still essential for drug susceptibility testing and improve the diagnostic yield for specimens.In this study it was aimed to determine the diagnosis of *Mycobacterium tuberculosis* infection rates at the patients followed by tuberculosis suspected in the hospitals where we serve, and compare the performance of the BACTEC MGIT 960 in fully automatic system with Lowenstein-Jensen medium.**Methods:** A total of 5548 specimens obtained from 2978 patients were cultured in parallel. Whose cultures were retrospectively evaluated from January 2017 to December 2017 from 13 hospitals at the the Central Tuberculosis Laboratory of Istanbul Northern Anatolian Association of Public Hospitals. **Results:** Of the 5548 specimens included in the study obtained from 2978 patients were cultured. 91% of diagnostic cultures turned positive within 14 days. 79% of them being represented by M.tuberculosis complex. The best yield was obtained with the BACTEC MGIT 960 (Beckton-Dickinson, USA) system with 405 isolates. To comparison with 405 isolates with the BACTEC MGIT 960 system, 374 isolates obtained with Lowenstein-Jensen medium in parallel cultures. The shortest times to detection were obtained with the BACTEC MGIT 960 system (10.7 days average); 14 days earlier than that with Lowenstein-Jensen medium (24.7 days average) . The BACTEC MGIT 960 system

had a contamination rate of 7%, Lowenstein-Jensen medium 12%. The best yield was obtained with the BACTEC 960 system, with 405 isolates, in comparison with 405 isolates with the BACTEC MGIT 960 system and 374 isolates with LJ medium. **Conclusion:** BACTEC MGIT 960 system is a fully automated, nonradiometric instrument that is suitable for the detection of growth of tuberculosis and other mycobacterial species and that is characterized by detection times that are even shorter than LJ medium. A fast and reliable diagnostic method that would differentiate between active and latent TB infection is also lacking.

B-096

Use machine learning-based approach to analyze MALDI-TOF MS data for a rapid and accurate reporting MRSA

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Background: Early discriminating Methicillin resistant *Staphylococcus aureus* (MRSA) from methicillin sensitive *Staphylococcus aureus* (MSSA) could direct correct antibiotics administration. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) may provide early report of antibiotics susceptibility than conventional method. However, detecting antibiotics resistance by using massive data of MALDI-TOF MS has not been widely validated yet. A machine learning (ML)-based approach could serve as a potential tool in analyzing MALDI-TOF MS data for a rapid and accurate reporting MRSA. **Methods:** Two cohorts of *S. aureus* isolates were consecutively collected from clinical specimens in two distinct teaching hospitals. The isolates were analyzed by MALDI-TOF MS to obtain mass spectra. Determination of MSSA or MRSA was performed by disc diffusion. For applying ML, binning method was used first to standardize the peaks of mass spectra. Two feature selection methods, Pearson correlation coefficient (PCC) and One Rule were applied for selecting robust peaks. Various ML algorithms, namely support vector machine, k-nearest neighbor, decision tree (J48), and Random Forest were trained by the training cohort. The performance was externally validated by the test cohort. **Results:** The training cohort contained 3990 cases (MRSA: 2017; MSSA: 1883), while the test cohort was composed of 2100 cases (MRSA: 972; MSSA: 1128) cases. The error window of binning method was set with 10 m/z for standardizing the peaks. To design the prediction models, 43 peaks were selected by PCC. Among the various ML algorithms, J48 model outperformed the others, exhibiting 77.92% accuracy, 74.8 sensitivity, and 81.3% specificity in distinguishing MRSA from MSSA. **Conclusion:** A rapid and accurate preliminary report of MRSA could be accomplished by using the ML-based methodology. Early administration of correct antibiotics against *S. aureus* may have benefit in preventing morbidity, mortality, and shorting length of stay.

B-097

Decreased Siglec-9 expression on natural killer cell subsetassociated with persistent HBV replication

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Background: Siglec-9 is a MHC-independent inhibitory receptor selectively expressed on CD56^{dim} NK cells. Its role in infection diseases has not been investigated yet. Here we studied the association of NK Siglec-9 with chronic hepatitis B (CHB) infection. **Methods:** Flow cytometry evaluated the expression of Siglec-9 and other receptors on peripheral NK cells. Immunofluorescence staining was used to detect Siglec-9 ligands on liver biopsy tissues and cultured hepatocyte cell lines. Siglec-9 blocking assay was carried out and cytokine synthesis and CD107a degranulation was detected by flow cytometry. **Results:** Compared to healthy donors, CHB patients had decreased Siglec-9^{+} NK cells, which reversely correlated with serum HBeAg and HBV DNA titer. Siglec-9 expression on NK cells from patients achieving SVR (sustained virological response) recovered to the level of normal donors. Neutralization of Siglec-9 restored cytokine synthesis and degranulation of NK cells from CHB patients. Immunofluorescence staining showed increased expression of Siglec-9 ligands in liver biopsy tissues from CHB patients and in hepatocyte cell lines infected with HBV or stimulated with inflammatory cytokines (IL-6 or TGF-β). **Conclusion:** These findings identify Siglec-9 as a negative regulator for NK cells contributing to HBV persistence and the intervention of Siglec-9 signaling might be of potentially translational significance.

B-098

Implementation of an Infectious Disease Cloud Based Epidemiology Network in the United States and South America

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Background: Real-time data collection of respiratory disease is important for understanding the spatiotemporal dynamics of disease transmission worldwide. United States (US) healthcare professionals use tools such as FluView to help identify local pathogen circulation; however, these tools are limited to syndromic surveillance, track a limited set of pathogens and do not typically span multiple continents. Understanding respiratory disease dynamics is facilitated by 1) a large, pathogen rich data set 2) geographically dispersed data sources, and 3) fine temporal resolution. Here we describe the expansion of the BioFire® FilmArray® Syndromic Trends (Trend), a research epidemiology system containing exported data from BioFire® FilmArray® Respiratory Panel (RP) tests, from the United States to Colombia, South America. **Methods:** Data from over half a million FilmArray RP tests have been exported to the Trend database from 30 labs across the United States since 2013. In 2017, Trend was implemented and tested in four clinical laboratories across Colombia, allowing test results to be automatically exported from these clinical laboratories to the centralized Trend database. The pathogen detection and co-detection rates from these data were then contrasted to trends observed in data from clinical laboratories in the United States. **Results:** The BioFire® FilmArray® Systems participating in Colombia exported a total of 1,400 test results to the Trend database, dating back to November of 2015, with a majority of tests obtained from archived data. Overall RP positivity rate of the Colombian tests was 65% (95% confidence interval 61-69) compared to 50% (95% confidence interval 49-50) for the US. Tests with multiple detections were similar, with 10% of Colombian tests being positive for more than one pathogen, in contrast to 7% in the US. Individual pathogen detection rates were similar for the two regions, with the exception of Respiratory Syncytial Virus (RSV), which accounted for 25% of all positives in Colombia, contrasted with 7% of positive US samples. In 2016, the predominant Influenza A serotype in both Colombia and the US was H1-2009. In 2017, the predominant serotype was H3 for both locations. Type H3 is currently the predominating serotype in the US with Colombia yet to be determined. The respiratory season in Colombia appears to have two peaks roughly six months apart: one in late spring, the other in late fall. The late fall peak is primarily associated with RSV. For US sites, the respiratory season typically peaks in January or February, with RSV peaking in December. **Conclusion:** BioFire SyndromicTrends shows great promise in deciphering spatiotemporal dynamics of common respiratory pathogens. This epidemiological system can identify global differences in disease dynamics overtime. Future work with finer geographic distribution of contributing sites will aid in making conclusions regarding spatial dynamics of all 20 RP pathogens.

B-099

Detection of clinically relevant and unusual uropathogens obtained from urine culture from patients in Rio de Janeiro.

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Background: Urine culture is the most routine specimen in Clinical Microbiology laboratories. Urinary tract infections (UTI) are caused by a wide variety of uropathogens. Usually the clinical diagnosis of UTI is confirmed with the result of the urine culture associated with the result of urine sedimentation. Occasionally, disagreement between the results of both exams occurs, with altered sedimentation and negative culture. Pesence of fastidious microorganisms is one of the explanations for this disagreement because they do not grow in culture media routinely used in urine culture. The present study evaluated the detection of clinically relevant pathogens in urine culture from patients with sedimentation positive for nitrite and with pyuria and negative cultures. **Methods:** : From March to December 2017, 406,942 urine cultures were obtained from several units spread throughout the State of Rio de Janeiro, Brazil. Among these 37,919 (9.31%) were positive. However, in 114 patients, the urine had a positive nitrite and pyuria with no growth of microorganisms at culture. All urine cultures are routinely processed on CLED agar with a calibrated handle of 103 and incubated at 35 ° C for 48 hours. For those 114 negative urine cultures with pyuria and positive nitrite, we recultured the urine on a chocolate agar plate with a calibrated loop of 102 and incubated at 35 ° C in CO2 atmosphere for 48 hours to investigate nutritionally fastidious bacteria. **Results:**A total of 29 microorganisms were obtained from the agar chocolate plate of the 114 urine recultured. Only one bacteria was isolated in each of these 29 speci-

mens. They were identified by automated mass spectrometry (Vitek MS MALDI-TOF) method. 20 isolates were *Haemophilus* spp., 6 *Gardnerella vaginalis*, 2 *Oligella urethralis* and 1 *Streptococcus pneumoniae.* **Conclusion:**Many urine cultures without isolation of microorganisms, with altered sedimentation and with clinical diagnosis of urinary tract infection may present unusual uropathogens. It is important for all Laboratory of Microbiology to evaluate these cases, aiming at the recovery of these uropathogens. It is important that the microbiologist assess the result of sedimentation and the culture, looking for inconsistency between the both exams and in this case, routinely use another culture media in order to identify fastidious bacteria. A total of 29/114 (25,4%) urines with discrepancies between sedimentation and culture results would have a false negative culture if there was no active research of fastidious microorganisms implanted in the laboratory routine.

B-100

Anti-CMV IgM Antibodies on Filter Paper: An Alternative Approach to Internal Quality Control in Neonatal Screening.

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Background: The objective of the study was to verify if blood samples collected on filter paper can be routinely used as additional control because the commercial kit does not bring the presentation of controls in the same matrix of the neonatal samples. In addition to the liquid controls of the kit itself, extra controls were used in the 275 trials over a year, samples in DBS being 11 Reactive and 7 negative. **Methods:** Anti-cytomegalovirus IgM antibodies were checked in neonatal screening routine on dried blood samples collected on filter paper (DBS, S&S903) using ELISA-Serion Classic automated immunoassay. The Optical Density was measured at wavelengths 405 and 620 at 690 nm on Immunomat machine. **Results:** see the table bellow **Conclusion:** Reactive samples have compromised stability when subjected to successive cycles of refrigeration and exposure to room temperature. The measurement of DOs gradually declines day by day with the time of use, usually from day 7 and in a variable way, with reflection in the final calculation, translating into "gray area", justifying the high CV found (53,53). By observing the ODs of the assays, our consensus was that samples in DBS can and should be used as an alternative to internal quality control of the kit, being important parameter of the test because they will express on the same matrix the variations that are submitted to all samples.

	Cytomegalovirus IgM - 275 dosages in DBS samples over 1 year in Neonatal Screening					
	by comercial kyt			DBS samples (3,2mm punch)		
	Expected mean STDs (reference)	STD obtained	Negative liquid control (by kit)	Non-Reactive DBS (by Baby samples)	Reactive DBS (by Baby samples)	Reactive DBS (in house sample)*
O.D. min	0,450	0,625	0,005	0,004	0,286	1,515
O.D. max	1,615	1,547	0,127	0,148	2,179	2,210
O.D. medium	0,931	1,124	0,048	0,050	0,773	1,875
SD	0,04	0,11	0,01	0,03	0,32	0,16
CV	4,14	9,52	25,09	56,2	53,53	8,77

(*) In addition, a Reactive sample made in house was used in filter paper (equal parts IgM reactive of serum for cytomegalovirus with red blood cell concentrate). This sample had the highest reactivity index.

B-101

Testing anti-Zika virus NS1 IgA additionally to IgM increases sensitivity in acutely infected patients from regions endemic for flaviviruses

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Background: Specific IgM response to Zika virus (ZIKV) can be low or absent in patients with acute ZIKV infection and a history of other related flavivirus infections, e. g. with Dengue virus (DENV), presenting with an early high IgG titer. In these ZIKV cases, IgA against ZIKV non-structural protein 1 (NS1) was observed in the acute phase, suggesting anti-ZIKV IgA as alternative acute marker in secondary infections. In this study, we investigated the diagnostic benefit of an ELISA for combined detection of anti-ZIKV NS1 IgA and IgM. **Methods:** The following human serum panels were included in this study: 1) A sensitivity cohort (cohort 1) comprising acute serum samples (day 8-16 post symptom onset) of 31 residents from Colombia (2015), where ZIKV and DENV are endemic. Patients had been tested positive for ZIKV nucleic acid and anti-DENV IgG during the viraemic phase ($\leq$ day 5). 2) A specificity cohort (cohort 2) consisting of serum samples (day 3-7 post symptom onset) of 40 Vietnamese patients, hospitalized with DENV hemorrhagic fever according to the World Health Organization case definition grade I and tested positive for DENV nucleic acid and anti-DENV IgG. Vietnam (2015) is endemic for DENV but not for ZIKV. Anti-ZIKV NS1 antibodies were determined in each sample using a commercial NS1-based Anti-Zika virus ELISA IgM (Euroimmun AG, Germany) and a corresponding ELISA (Euroimmun), applying a combination of anti-human IgA/IgM conjugated with peroxidase. **Results:** In cohort 1, 30 % (9/31) of samples were positive for anti-ZIKV NS1 IgM, whereas 100 % were positive for combined specific IgA and IgM. In cohort 2, none of the sera reacted in the Anti-Zika virus ELISA IgM, two samples were reactive in the Anti-Zika virus IgAM ELISA (5.0 %). **Conclusion:** Because patients with acute ZIKV infection from flavivirus endemic regions may not develop NS1-specific antibodies of class IgM, additional testing of anti-ZIKV NS1 IgA is required.

B-102

Multiplexed Host Response Biomarker Analysis on a Rapid, Quantitative Point-of-Care Platform

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Objective and Relevance. Literature suggests that host response biomarkers during acute infection may yield clinically relevant diagnostic or prognostic information. Rapid (< 30 min) detection of circulating protein biomarkers could provide actionable information during temporally complex conditions such as sepsis. Here we provide results for a rapid 3-plex host response marker assay run on a portable, point-of-care assay platform. The initial demonstration includes interleukin-6 (IL-6), procalcitonin (PCT), and C-reactive protein (CRP) in a single measurement. Data are presented for a collection of pediatric serum samples from patients clinically classified as sepsis, septic shock, and SIRS. **Methodology**. The MBio system consists of a disposable sample cartridge and portable reader for performing multiplexed fluorescence immunoassays. The cartridge-based assays combine a proprietary planar waveguide illumination approach with microarray-based spatial multiplexing and fluorescence imaging in a simple reader. The cartridge incorporates a fluidic channel with an array of capture antibodies. Workflow was as follows: each sample was mixed with a detection reagent comprising a cocktail of biotinylated antibodies and immediately added to the MBio cartridge. The mixture was incubated on-cartridge for 20 minutes, followed by a 10-minute streptavidin-fluorophore incubation. The IL-6 / PCT / CRP panel was selected to be representative of the range of host response markers that could be configured on the platform. Of note, we demonstrate simultaneous detection of a high concentration target (CRP > 30 micrograms/mL during inflammation) and a low concentration target (IL-6 limit of quantitation ~25 to pg/mL) in the same sample. **Clinical Sample Validation**. A collection of de-identified pediatric serum samples was provided by Dr. Hector Wong of the Cincinnati Children's Hospital. Samples were selected to include 10 from clinically identified pediatric sepsis patients, 10 SIRS, and 30 septic shock. Samples were run on the MBio platform, and reference ELISAs were performed for IL-6 and CRP. **Results**. Quantitative IL-6, PCT, and CRP results were generated on the MBio platform. There was overall correlation between MBio and the reference ELISAs. Three of 50 samples returned values beyond range (high) for the ELISA and MBio assays. Four samples were below detection limit for IL-6 on MBio. Most samples in the collection showed high PCT (> 0.5 ng/mL), as expected. 29 of 30 samples from septic shock patients showed PCT well above threshold. The one low PCT sample in this set showed significant hemolysis which may have affected the MBio result. The SIRS samples were also elevated in PCT, but were much less likely to be above threshold. These results suggest that the MBio assay is detecting differences in these clinically distinct categories. The CRP assay showed correlation with ELISA, but there were several sample with significant quantitative differences suggesting the CRP assay needs further optimization. CRP does not appear to be a discriminatory marker for the three clinical categories. **Conclusions**. Preliminary clinical sample data for this 3-plex assay suggest that the MBio platform can be used to deliver quantitative, protein biomarker panel results on clinically relevant samples in less than 30 minutes.

B-103

Detection of cytomegalovirus nucleic acid and mycoplasma nucleic acid in alveolar lavage fluid of pediatric patients with respiratory tract infection

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Background: There are limited reports on mixed infection of common atypical pathogens, so this study investigated the infection of human cytomegalovirus (HCMV) and mycoplasma pneumoniae (MP) in alveolar lavage fluid of pediatric patients with respiratory tract infection. **Methods:** A total of 31 pediatric patients with respiratory tract infection were enrolled in the Department of Pediatrics, the First Affiliated Hospital of Anhui Medical University from May to August in 2017, and the HCMV nucleic acid in the alveolar lavage fluid was detected by real-time fluorescent polymerase chain reaction. Ribonucleic acid (RNA) thermostatic amplification technology was used to detect MP nucleic acid in the alveolar lavage fluid. **Results:** The total detection rate of both pathogens was 64.52%, the positive rate of HCMV nucleic acid was 38.71%, and the positive rate of MP was 25.81%. **Conclusion:** HCMV and MP have high infection rate in pediatric patients with respiratory tract infection. The combined detection of these two infectious agents in alveolar lavage fluid has important application value for clinical etiology and treatment.

B-104

The mutable profile of infectious Candida species and resistance to antifungal agents: a clinical and laboratorial study

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Background: Vulvovaginitis by Candida spp, or vulvovaginal candidiasis CVV, is a common infection whose symptoms, located in the vulva and vagina, are characterized by intense pruritus and thick vaginal discharge, sometimes forming gums that adhere to the surface of the mucosa. This infection most often affects women of reproductive age throughout the world. However, the literature data on its incidence are incomplete because it is a non mandatory notification infection and because of the inaccuracy of the frequently used clinical diagnosis. To identify *Candida* species in patients with vulvovaginitis, determining their sensitivity to antifungal agents. **Methods:** were analyzed 84 vaginal secretion samples of patients seen at the Brasilia University Hospital Gynecology outpatient clinic. Nineteen patients were asymptomatic and 65 with vulvovaginitis, disclosing at least one of the following symptoms: vaginal discharge, vulvar hyperemia or edema, and localized itching or burning sensation. *Candida* phenotype was identified by culture, and confirmed by Matrix Assisted Laser Desorption Ionization Time-of-flight MALDI TOF. The sensitivity profile of *Candida* ssp for fluorocytosine, fluconazole, voriconazole, amphotericin B, capsofungin and mycofungin was determined by the Minimal Inhibitory Concentration MIC. **Results:** sample analysis of the 65 symptomatic patients showed 73% 48 positivity, with 75% 36 of the phenotypes identified as *Candida albicans*, 22.9% 11 as non-albicans species respectively, 8.3% of *C. glabrata*, 6.2% of *C. parapsilosis*, 4.2% of *C. tropicalis*, 2.1% of *C. krusei*, 2.1% of *C. Zeylanoides* and 2.1% of *Rhodotorula mucilaginosa*. In the antifungigram showed that *C. albicans* species were sensitive to all antifungal with the exception of one of the species that showed an intermediate sensitivity to amphotericin B 2.1%. Resistance was found among non-albicans species to fluconazole in 2.1% *C.glabrata*, to fluconazole in 2.1%, and to voriconazole in 2.1% *C. Krusei*. **Conclusion:** In view of significant increased infectivity of non-albicans species, with some phenotypes already showing resistance to usual antifungal agents, our results emphasize the need to precisely identify the *Candida* species, in order to abrogate possible treatment failure and repetitive episodes of vulvovaginitis.

B-105

Nucleic Acid Capture Using Silicon Dioxide Derivatized Magnetic Particles Provides the Foundation for Sensitive and Precise High Throughput Automated RT-PCR Assays

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Background: To address the need for nucleic acid capture in downstream and high throughput applications targeting the detection of viruses, we sought to develop magnetic particles (for research use only, not for use in diagnostic procedures) that would provide desirable analytical performance characteristics (limit of detection (LoD)), precision, and linear range) in automated RT-PCR assays and could be produced in large quantity in our own lab. **Methods:** Silicon-coated magnetic particles with a proprietary functionalization group were manufactured in our lab according to best practices. Approximately 1 milligram of magnetic particles are used per test to capture nucleic acids of lysed organisms. Each test was performed using K_2EDTA plasma or serum. Approximately 300 samples were collected and along with the particles, were loaded on to a fully automated processing instrument. Sample introduction, nucleic acid extraction, real-time PCR (RT-PCR) for HBV, HIV, HCV and CMV reaction setup, amplification and purification were performed without manual intervention. **Results:** The LOD results were 18 IU/mL for CMV in K_2ETDA plasma, 2.0 IU/mL for HBV K_2ETDA plasma and 3.8 IU/mL HBV in serum, 4.3 IU/mL for HCV and 30 IU/mL for HIV-1 both in K_2ETDA plasma. The standard deviation of the precision (Log IU/mL) was less than or equal to 0.16 for HBV, 0.15 for HCV, 0.16 for CMV and 0.20 Log cps/mL for HIV. The linear range (Log IU/mL) was 2.00-7.01 for CMV, 1.00-9.00 for HCV, 1.5-6.4 for HCV and 1.32-6.8 for HIV. **Conclusion:** The functionalized magnetic particles provide efficient DNA/RNA capture for high throughput detection and amplification of viral RNA using RT-PCR in a completely automated system. The extracted DNA/RNA from serum and plasma provides the basis for assays that have desirable performance characteristics that include: sensitivity, limit of detection, precision and linear range. We are currently evaluating the large scale production of the particles (0.6 Kg batches) and the use of the particles in other high throughput applications that require robust RNA/DNA extraction.

B-106

A Machine Learning Approach to Inflammatory Cytokine Profiling Reveals Diagnostic Signatures for Latent Tuberculosis Infection and Reactivation Risk Stratification

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Background: Latent tuberculosis infection (LTBI) is estimated in nearly one third of the world's population, and of those infected 10% will proceed to active tuberculosis (TB). Current diagnostics cannot definitively identify LTBI and provide no insight into reactivation risk, thereby defining an unmet diagnostic challenge of incredible global significance. However, by leveraging the unique immunological response to TB, a signature of cytokines may be useful for LTBI diagnostics. **Methods:** Using a silicon photonic microring resonator array, we developed and analytically characterized a 7-plex cytokine assay capable of automated screening of subject signatures in 46 minutes. This panel was used for profiling secreted immune response in LTBI-relevant samples from a 50-subject cohort with variable TB exposure risk. Peripheral blood mononuclear cells (PBMC) were isolated and immunologically challenged with five different stimulation conditions including two TB-specific antigens (CFP-10/ESAT-6, PPD) as well as three different controls representing positive (CD-3), negative (media), and off-target (*candida albicans*) immunological response. The panel of cytokine biomarkers was then quantitated in the supernatant of PBMCs from each stimulation condition. Additionally, all subjects were assessed for LTBI status and reactivation potential through standard-of-care diagnostic tests and regulatory guided classification. Absolute and control normalized responses for each biomarker (i.e. CD-3 stimulated response subtracted from CFP-10/ESAT-6 response) were evaluated for improved diagnostic capabilities using a machine learning guided feature selection algorithm. **Results:** Detection limits typically below 10 pg/ml, quantitation limits below 200 pg/ml, and inter-assay CVs at or below 10% were achieved with comparable response to ELISA. Boruta feature selection identified stimulated biomarker features that are predictive for LTBI and reactivation risk diagnoses. Normalized features, aiming to correct for differences in the basal immune state of each individual, were statistically revealed as unique from related stimulated responses and predictive for LTBI relative to healthy subjects. Notably, largely consistent signatures were identified for subjects with CDC defined LTBI as well as a stricter LTBI definition with IFN-ɣ, IP-10, IL-2, and CCL4 (under different combinations of stimulation normalization) showing strong predictive correlations. Orthogonal biomarker signatures were found to correlate with high and low reactivation risk. **Conclusions:** We developed and validated a multiplexed immunodiagnostic approach toward diagnosis of LTBI and stratification of reactivation risk that relies entirely upon secreted biomarker signatures from a simple in vitro assay. Multiplexed cytokine detection from within patient-derived samples of TB-related antigen exposure was performed using a silicon photonic platform that showed robust analytical performance. The biomarkers IFN-ɣ, IP-10, IL-2 appear as particularly promising markers for assessing LTBI status and TB reactivation risk when considered in light of comprehensive stimulation conditions and precision normalization for heterogeneities in basal immune response.

B-107

Comparison of clinical performance of SD Strep A Ultra Test and SD Strep A Rapid Test for diagnosis of acute bacterial pharyngitis

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Background: Rapid and accurate diagnosis of bacterial pharyngitis is essential for the optimal antibiotic treatment. Clinical performance of SD Strep A Ultra test (SD, Korea), a recently developed rapid antigen detection test (RADT), was evaluated for children with pharyngitis. **Methods:** Three-hundred forty three children with sore throat visiting seven pediatric clinics in Changwon, Korea were subjected to throat swabs twice during April-September, 2017. The first flocked swab was used for SD Strep A Ultra test. The other two cotton swabs were used for SD Strep A Rapid test and culture. PCR detecting *speB* gene was carried out for RADT-positive and culture-negative specimens. Clinical performance of SD Strep A Ultra was analyzed by the colony numbers and color intensity (range 1-20). The colony numbers were defined as 1+ for <10 CFU, 2+ for 10-50 CFU, 3+ for 51-100 CFU, 4+ for >100 CFU. This study was approved by IRB of Changwon Changwon Gyeongsang National University Hospital and all participants agreed on written consent. **Results:** Sensitivity, specificity, positive predictive value, and negative predictive value of SD Strep A Ultra were 97.4%, 90.8%, 93.0%, and 96.5%, respectively and those of SD Strep A Rapid were 95.8%, 94.7%, 95.8%, and 94.7%, respectively compared to throat culture. All three specimens showing RADT-positive and culture-negative were positive for the *speB* gene. When comparing with colony numbers, SD Strep A Ultra was negative with a frequency of 14.3% of 1+, 0% of 2+, 5.0% of 3+, and 0.9% of 4+ ($P = 0.021$). When comparing with the color intensity of SD Strep A Ultra, the frequency of GAS-negative was 11.5%, 15.9%, 3.9%, and 0% in the ranges of 1-5, 6-10, 11-15, and 16-20, respectively ($P < 0.001$). Area of ROC curve was 0.938 for the evaluation of diagnostic accuracy with color intensity of SD Strep A Ultra test. **Conclusions:** SD Strep A Ultra exhibited an excellent sensitivity and negative predictive value and comparable performance with SD Strep A Rapid. Discrepant result with culture might be due to different swab material (flocked swab and cotton swab), sampling order, bacterial numbers of GAS, and delayed transport.

B-108

Standardization of new indirect ELISA using a highly-specific egg protein from *Schistosoma mansoni* for diagnosis of different clinical forms in a low endemic area in Brazil

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Background: Schistosomiasis remains a global public health problem. In 2012, the WHO declared the elimination goal by 2020 and emphasized the need to develop highly accurate diagnostic tools adapted to low endemic areas. In Brazil the disease is caused by the species *Schistosoma mansoni* and is characterized by chronic low-intensity infections (<100 egg per gram of feces) in endemic areas and acute cases derived from internal migration and tourism. The “gold standard” method for WHO guidelines is the Kato-Katz, a stool microscopy-based technique which has low sensitivity in endemic areas of Brazil. In order to develop more sensitive tests, we searched for a specific marker and standardized by a conventional technique, enzyme-linked immunosorbent assay (ELISA). As a long-term goal, we intend to apply this marker on innovative technologies feasible to be used in low resource areas in a test-and-treat format. **Methods & Materials:** Using a protocol approved by the Brazilian Ethical Committee (n. 893.582), human serum was obtained from each group: healthy volunteers (negative controls); schistosome acute, chronic and post-treatment patients; and patients infected with other helminths. Fifteen samples from each group were pooled and submitted to two-dimensional Western blot (2D-WB) using native and sodium metaperiodate (SMP) treated schistosome soluble egg extract (SEA). The immunoreactive spots were identified by mass spectrometry and analyzed by bioinformatics tools. Recombinant protein of the selected biomarker was produced and applied to development of indirect ELISA using serum samples. **Results:** A total of 23 spots were identified. Among these, 22 spots were identified by serum from patients infected with other helminths, and 10 by negative control samples. Only 1 spot was recognized by *Schistosoma*-infected patients and detection remained after sugar denaturation by SMP. We identified this sequence (Major Egg Antigen), cloned using Gateway methodology, and produced the recombinant protein. The antibody detection by ELISA showed 88% sensitivity and 66% specificity in serum from Brazilian low-intensity infections. The next steps will be (1) ELISA evaluation using serum from different Brazilian endemic areas, (2) standardization using non-invasive samples to assess functionality, (3) production of monoclonal antibodies and evaluation of direct detection, and (4) development of a new point-of-care assay. **Conclusion:** The development of a new diagnostic requires long-term investments and we successfully achieved the initial steps. We identified a highly specific egg protein and showed its good performance in conventional ELISA making it promising candidate for improving Schistosomiasis diagnosis. We intend to develop innovative immunological methods using non-invasive samples as required by public sector. Furthermore, a test that is easier to use in the field will improve the accuracy for mapping of areas, to monitor impact strategies and perform post-elimination surveillance. We believe these new assays can potentially achieve the WHO guidelines and be included in elimination strategy programs used in affected countries.

B-109

Same-day checkup for active type of *Mycobacterium tuberculosis* complex by ultrasensitive ELISA

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Background: The definitive diagnosis has been believed to be performed with for tuberculosis. However, PCR detects not only active *Mycobacterium tuberculosis* (TB) complex but also nucleic acids obtained from dead TB. To fight tuberculosis, a rapid checkup for active type of TB complex is crucial. Recently, we have developed an ultrasensitive ELISA to detect proteins at 10^{-20} moles/test by use of enzyme cycling, in which a cycling reaction is conducted by a dehydrogenase (3α-hydroxysteroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androsterone derivatives). In the present study, we applied this ultrasensitive ELISA to a checkup for the active type of TB complex. Our proposed method provides the same-day (4-hour) results. Methods: We used MPB64, a specific protein secreted from active TB complex as a biomarker. BCG was used for the TB complex, and it was added into sputum obtained from people without tuberculosis. As a pre-treatment for the ultrasensitive ELISA, we warmed up BCG in the sputum and enhanced the secretion of MPB64. In the sandwich ELISA, two specific antibodies for MPB64 were used, one of which was conjugated with alkaline phosphate (ALP). An androsterone derivative with a phosphate was hydrolyzed by ALP, and this derivative was then employed in the enzyme cycling. Consequently, MPB64 could be determined by the accumulated amount of thio-NADH in the enzyme cycling. Results: The spike-and-recovery test using BCG and sputum demonstrated reasonable results. We succeeded in detecting TB (i.e., BCG) in the sputum at the level of 3×10^2 CFU/mL within only 4 hours. This rapidity can contribute to the prevention of disease spread, because potential patients can be isolated during the 4 hours that the results take. The present available tests for active TB detection are the sputum smear test and the sputum culture test. The smear test has low sensitivity (> 10,000 CFU/mL), whereas the culture test is highly sensitive (tens to hundreds CFU/mL) but requires a long culture period (at least 10 days). Furthermore, we applied our ultrasensitive ELISA to the sputum collected from the tuberculosis patients who had been already diagnosed with a BD BACTEC MGIT 960 Mycobacteria Culture System. The comparison results showed that the positive conformity ratio was 89% and that the negative conformity ratio was 98%. That is, the total conformity ratio was 95%. Conclusion: The present results showed that our ultrasensitive ELISA can be used enough to detect active TB complex within 4 hours. A conventional nucleic acid amplification test may detect dead bacteria, and it sometimes shows a false negative because of a small amount of bacteria in the sputum. Our present method, on the other hand, is a user-friendly ELISA without any specialized apparatus: it has almost the same sensitivity as the culture method but with same-day results. We believe that the detection of active TB complex within 4 hours enables us to judge the therapeutic effects.

B-110

Performance characteristics of the Alinity i HBsAg Qualitative II, Anti-HBc II and Anti-HBs assays utilized for routine laboratory Hepatitis B testing

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Background: Alinity i is a compact immunoassay system and a member of Abbott's next generation family of laboratory analyzers. Routine diagnosis of Hepatitis B Virus (HBV) infection is often assessed by using a panel consisting of HBsAg, Anti-HBc and Anti-HBs assays. The aim of the current study was to evaluate the key performance characteristics of these three assays that were developed for the Alinity i system. **Methods:** The Alinity i HBsAg Qualitative II, Anti-HBc II and Anti-HBs assays were tested side by side with the corresponding ARCHITECT assays. Analytical sensitivity for HBsAg, and Anti-HBc was determined using the corresponding WHO standards. A study to determine Limit of Blank (LoB) / Limit of Detection (LoD) / Limit of Quantitation (LoQ) for Anti-HBs was performed based on guidance from CLSI EP17-A2, whereas the measuring interval was determined based on guidance from CLSI EP06-A. Clinical specificity was assessed using unselected blood donor and routine diagnostic specimens, clinical sensitivity was determined using pedigreed positive specimens. **Results:** The analytical sensitivity of the Alinity i HBsAg Qualitative II assay was determined to be 19.93 - 20.87 mIU/mL (WHO 2nd IS, NIBSC code: 00/588). The Anti-HBc II assay exhibited an analytical sensitivity of 0.54 - 0.56 IU/mL on the WHO 1st IS (NIBSC code: 95/522). The clinical sensitivity of the Alinity i HBsAg Qualitative II assay was found to be 100,00 % using 496 known positive samples including different genotypes and mutants. The Alinity i Anti-HBc II assay also showed 100,00 % sensitivity, detecting all specimens from patients with acute, chronic and past/resolved HBV infection with anti-HBc antibodies. The specificity for blood donor specimens of the Alinity i assays under evaluation was 99.96% (5108/5110) for HBsAg Qualitative II and 99.86% (5162/5169) for Anti-HBc II. Similar values were found for the corresponding ARCHITECT assays (99.96% and 99.88%, respectively). Diagnostic specificity was found to be 100,00 % for HBsAg Qualitative II and Anti-HBc II on the Alinity i as well as on the ARCHITECT platform. The quantitative Alinity i Anti-HBs assay, standardized to the WHO 2nd International Reference Preparation, 2008 (code 07/164), had a LoB of 0.53 mIU/mL, LoD of 0.77 mIU/mL, and a LoQ of 2.00 mIU/mL. It showed performance within acceptance criteria for linearity, imprecision, and bias across the entire measuring range from 2.00 mIU/mL up to 1000.00 mIU/mL. Quantitative correlation between Alinity i and ARCHITECT Anti-HBs assays exhibited a slope of 1.08. **Conclusion:** The key performance characteristics of the three Alinity i assays used for routine Hepatitis B testing, HBsAg Qualitative II, Anti-HBc II and Anti-HBs are equivalent to the corresponding ARCHITECT assays. This will enable easy transition of existing ARCHITECT customers to the new Alinity i system that offers state of the art technology for increased operational efficiency.

B-111

Quantitative Determination of Procalcitonin (PCT) In Human Serum by Lumipulse®G B•R•A•H•M•SPCT Assay

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INTRODUCTION: PCT (procalcitonin), a precursor of calcitonin, is synthesized by C-cells in the thyroid under normal conditions. Systemic inflammatory responses triggered by severe bacterial infections or sepsis, significantly increases synthesis of PCT resulting in elevated serum and plasma PCT levels. PCT is induced more strongly by bacterial infections compared to other inflammatory reactions (i.e. viral infections, autoimmune disease, transplant rejection, allergic reactions). Therefore, *in vitro* determination of PCT can be used to formulate differential diagnosis or to indicate severity of severe bacterial infections and sepsis. **METHODS:** The Lumipulse G B•R•A•H•M•S PCT is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of PCT in specimens on the LUMIPULSE G System by a two-step sandwich immunoassay method. PCT specifically binds to an anti-PCT monoclonal antibody (mouse) and anti-calcitonin monoclonal antibody (mouse) coated on particles and forms immunocomplexes. After washing, an alkaline phosphatase (ALP: calf)-labeled anti-katacalcin monoclonal antibody (mouse) specifically binds to PCT immunocomplexes, completing the sandwich. The amount of PCT is derived from the luminescence signals generated by adding the substrate AMPPD (3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt). Calibration of the Lumipulse G B•R•A•H•M•S PCT assay are traceable to in-house reference calibrators whose values have been assigned to Thermo-Fisher Scientific Inc.'s B•R•A•H•M•S PCT sensitive Kryptor. All verification and validation studies were performed according to respective CLSI guidelines. **RESULTS:** The Limit of Blank, Limit of Detection and Limit of Quantitation of the Lumipulse G B•R•A•H•M•S PCT assay was ≤0.0114 ng/ml. The Lumipulse G B•R•A•H•M•S PCT assay demonstrated linearity in the range from 0.010 to 104.260 ng/ml. There was no high-dose hook effect observed for samples containing up to ~12,000 ng/ml of PCT. A twenty-day precision study of 8 human serum-based panels and two commercially available serum-based controls assayed in duplicate at two separate times of the day using two LUMIPULSE G1200 systems (n = 80 for each sample) demonstrated within-laboratory (total) precision of ≤ 4.7%. Interference studies demonstrated an average difference of ≤ 10% between control and test samples containing potential interfering compounds, including 9 endogenous substances (free bilirubin, conjugated bilirubin, triglycerides, hemoglobin, human serum albumin, immunoglobulin G, biotin, human anti-mouse antibody, and rheumatoid factor) and 26 commonly used therapeutic drugs. Cross- reactivity of the Lumipulse G B•R•A•H•M•S PCT assay with other substances (Human Calcitonin (10 ng/ml), Human Katacalcin (10 ng/ml), α-CGRP (10,000 ng/ml), β-CGRP (10,000 ng/ml), Salmon Calcitonin (13.2 μg/ml), and Eel Calcitonin (7.5 μg/ml), respectively) that are similar in structure to PCT demonstrated no cross-reactivity. A comparison of Lumipulse G B•R•A•H•M•S PCT with a FDA-cleared predicate device was analyzed using weighted Deming regression. For the 207 tested specimens (concentrations ranged from 0.054 to 58.156 ng/ml), the slope, y-intercept, and correlation coefficient (r) were 1.0199, -0.0044, and 0.9535, respectively. In a population of 213 self-reported healthy individuals, the 95th percentile, upper reference range limit was calculated at 0.045 ng/ml. **CONCLUSIONS:** The data demonstrate that the Lumipulse G B•R•A•H•M•S PCT assay on the automated LUMIPULSE G1200 System is sensitive, accurate and precise for routine quantitative determination of PCT in serum and plasma specimens.

B-112

TLR1polymorphismsare significantly associatedwith the occurrence, presentation and drug-adverse reactions oftuberculosis in Western Chinese adults

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Background: Obtaining further knowledge regarding single nucleotide polymorphisms (SNPs) in the *TLR1* gene is of great importance to elucidate immunopathogenesis and management of tuberculosis (TB). **Methods:** We enrolled 646 tuberculosis patients and 475 healthy controls from West China. Six SNPs in *TLR1* were genotyped in every individual and were analyzed for their association with TB susceptibility and clinical presentation. The prospective follow-up was performed to determine whether these SNPs are associated with adverse reactions to anti-TB drugs. **Results:** Rs5743565 and rs5743557 were significantly associated with reduced predisposition to TB regarding the mutant allele in additive and dominant models with odds ratios (ORs) ranging from 0.61 to 0.83. There was increased tuberculosis risk associated with the haplotype CAG (rs4833095/rs76600635/rs5743596) [OR (95% CI) = 1.33 (1.07-1.65), p = 0.009] and with haplotype GG (rs56357984/rs5743557) [OR (95% CI) = 1.21 (1.02-1.43), p = 0.029]. The erythrocyte and hemoglobin levels were significantly higher in TB patients with the rs5743557 GG genotype than for AA and/or AG genotype carriers (p = 0.006 and 0.020, respectively). Chronic kidney damage and hepatotoxicity were common side-effects with RIF and INH regimens in this study with occurrence rates of 21.56% and 10.32%, respectively. Rs5743565 seemed to pose a higher risk of anti-TB-induced hepatotoxicity under the dominant model [OR (95% CI) = 2.17 (1.17-4.05), p = 0.013], and rs76600635 GG/AG genotypes were clearly correlated with the development of thrombocytopenia [OR (95% CI) = 2.98 (1.26-7.09), p = 0.010]. **Conclusions:** Rs5743565 and rs5743557 in the *TLR1* gene may contribute to decreased risk for tuberculosis susceptibility in a Western Chinese population. Rs5743565 and rs76600635 are potential risk factors for adverse reactions to anti-TB drugs. Our data help to characterize the development and progression of TB disease in China; however, multicenter studies and research into the precise mechanisms are needed to confirm these results.

Keywords: tuberculosis; Toll-like receptor 1; single nucleotide polymorphisms; anti-TB drugs; adverse reactions; Western Chinese population

B-113

Clinical Performance of the Bio-Rad BioPlex 2200 Toxoplasma gondii IgM assay

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Background: *Toxoplasma gondii* is a parasite that can be acquired following ingestion of cysts from feces of infected cats, or from eating undercooked or contaminated meat. Toxoplasmosis is routinely diagnosed through detection of *T. gondii*-specific antibodies. A major problem with *T. gondii*-specific IgM testing is lack of specificity, resulting in false positive IgM results. The BioPlex 2200 ToRC assays (IgM and IgG; Bio-Rad Laboratories, Hercules, CA) are multiplex flow immunoassays intended for identification of antibodies to *T. gondii*, Rubella and CMV in human serum or plasma. The BioPlex 2200 ToRC IgM assay is a new formulation that received FDA clearance in May 2017. Here we sought to evaluate the clinical performance of the *T. gondii* IgM portion of this assay. **Methods:** Two sample populations were utilized: 1) Prospective: 300 consecutive residual sera submitted for anti-*T. gondii* IgG and IgM testing as part of routine clinical care; 2) Archived: 52 residual sera previously positive for anti-*T. gondii* IgM and IgG using the predicate ADVIA Centaur *Toxoplasma* assays (Siemens, Malvern, PA). Performance of the BioPlex 2200 ToRC IgM and IgG assays was evaluated by calculating positive percent agreement (PPA) and negative percent agreement (NPA) compared to the Centaur tests. **Results:** Among the 300 prospective specimens the BioPlex 2200 assay demonstrated a percent negative agreement (NA) and positive agreement (PA) of 99.3% (288/290, 95% CI: 98.3-100%) and 0% (0/7), respectively, with the Centaur assay. Review of the medical record revealed that the 7 Centaur *T. gondii* IgM positive samples in this population were likely false positives. IgG demonstrated 95.8% (251/262, 95% CI: 93.4-98.2%) NA and 82.3% (28/34, 95% CI: 69.5-95.2%) PA in this population. Among the 52 archived samples positive for both IgG and IgM by the predicate method, the BioPlex 2200 IgM and IgG assays demonstrated a 90.4% (47/52; 95% CI, 82.3-98.4%) PA and 100% (52/52) PA, respectively. **Conclusions:** The BioPlex 2200 *T. gondii* IgM demonstrated excellent concordance with the ADVIA Centaur assay and may deliver fewer false positive results in a low prevalence population.

B-114

Clinical performance of the Bio-Rad BioPlex 2200 Syphilis Total and RPR assay

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Introduction Syphilis infection caused by the spirochete, *Treponema pallidum*, is a major cause of sexually transmitted infections worldwide. Historically, serologic methods for the diagnosis of syphilis included a combined approach for the detection of antibodies to non-treponemal (RPR test) and treponemal (FTA-ABS or TP-PA test) antigens that are simple and reproducible but labor intensive. The BioPlex 2200 Syphilis Total and RPR assay (Bio-Rad Laboratories, Hercules, CA) was recently FDA-cleared and is a fully automated method for the simultaneous detection of treponemal and non-treponemal antibodies. Our objective was to evaluate the diagnostic performance of this assay at a tertiary medical center with a high rate of syphilis. **Methods** The study population consisted of 400 prospectively collected remnant serum specimens sent for syphilis testing as part of routine clinical care and 100 retrospectively collected RPR-positive specimens. Concordance of the BioPlex 2200 Syphilis Total & RPR assay to the predicate method was evaluated. The predicate method consisted of the Wampole RPR Card test with confirmation by the Inverness FTA-ABS test. Discrepant results were further tested using the Fujirebio Serodia TP-PA test. A titer was determined in any specimen positive by RPR. **Results** Of the 400 prospectively collected specimens, 263 (66%) were from females, of which 36 (14%) were pregnant, 166 (63%) were not pregnant, and 61 (23%) were of unknown pregnancy status. In total, 30 (8%) specimens were from HIV positive patients and the majority (81%) were 18 years of age or older. The positive and negative percent agreement (PPA and NPA) of the 400 prospectively collected specimens was 85% (17/20, 95% CI 84.5-85.5%) and 98% (373/380, 95% CI: 98.1-98.2%), respectively. The total concordance of the RPR results in the prospective population was 97.5% (390/400, 95% CI: 96-99%). Of the 3 potential false negative BioPlex RPR results, one specimen tested negative by both the confirmatory FTA-ABS predicate method and by TP-PA during discrepant analysis, suggesting that the negative BioPlex RPR result was true. Thus, the final result interpretation after confirmatory testing was 99% concordant (398/400) with the predicate method. The PPA of 100 predicate RPR positive retrospective samples was 88% (88/100, 95% CI: 87.8-88.2%). Of the 12 potentially false negative BioPlex RPR results, discrepant analysis by TP-PA revealed that 2 were likely falsely positive by the predicate FTA-ABS method. Seven of the 10 remaining potential false negative results were low positives with RPR titers < 1:4 by the predicate RPR method. For specimens with RPR titers determined using both the predicate and test method, there was a 79% agreement of the RPR titer within +/- one doubling dilution. **Conclusions**The performance of the BioPlex 2200 Syphilis Total and RPR assay was comparable to the predicate RPR and FTA-ABS methods. The high NPA of this assay, in combination with the ability to automate a historically labor intensive, make it well suited for use as a screen for syphilis in a high volume laboratory.

B-115

Evaluation ofFilmarray for Early Diagnosis of Sepsis

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Background: Bloodstream infections (BSI) are one of the most important causes of death in healthcare settings. Accurate and rapid methods for the diagnostic of these infections are crucial for patient´s survival. Bacterial resistance is a major concern in these patients especially in nosocomial BSI **Methods:** We evaluated the platform Biofire Film Array (BioMérieux - Marcy I'Étoile - France) using the blood culture identification (BCID) panel for the diagnostic of BSI. This PCR based method is performed using positive blood culture bottles for identification of 19 bacteria, 5 species of *Candida* and also 3 resistance genes targets. We compared this method with conventional blood culture and Mass Spectrometry (MALDI TOF - MT) for identification of pathogens directly from positive bloodcultures. A total of 45 cases were selected. We carried out the tests at the same time and compared: the agreement of pathogens identification, detection of resistance genes and turnaround time (TAT) for the results **Results:** For identification, we found 84.5% and 78.0% of agreement when compared Biofire and MT with conventional culture, respectively. The Biofire missed one case (negative result) whose culture showed growth of *Roseomonas* sp. Partial agreement occurred in 3 cases: one the Biofire identified only *Enterobacteriacea* gender and the culture was positive for *Citrobacter* freundii; two cases there were growth of multiple agents and Biofire identified only one. Invalid results were observed in 3 cases (2 *E. coli* and one *Pseudomonas aeruginosa*). On the other hand, MT showed partial agreement in 2 cases where only one agent was identified and the cultured was positive for multiple agents. Invalid results were observed in 8 cases. For the resistance genes, Biofire identified a *mec*A gene in a *S. aureus* but in the culture oxacillin susceptible. Concerning about the time, Biofire and MT presented similar TAT for identification, significantly shorter compared to conventional culture. **Conclusion:** we conclude that Biofire presented an excellent performance for identification and detection of resistance genes. The limitation of Biofire is identification of agents no present in the panel. For resistance genes the Biofire provided a good correlation with the final susceptibility testing for the genes targeted.

B-116

High Diversity of Yeasts Identified by MALDI TOF Mass Spectrometry in the Routine Clinical Microbiology Laboratory

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Background: Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has become a powerful tool for identification of pathogens, especially non-albicans Candida in clinical microbiology laboratories. The aim of this study was to evaluate the Vitek MS system (bioMérieux) as fast and reliable method for yeasts identification. **Methods:** From January to December 2017 we analyzed 18.854 fungi culture recovered from several clinical samples, including blood, peritoneal fluid, bronchoalveolar lavage, urine, wound and body fluid cultures. Clinical samples were cultivated first on Mycosel and Sabouraud-glucose agar, and then incubated at room temperature. All the isolates were identified by MALDI-TOF mass spectrometry using the Vitek-MS System which contains the MYLA database, according to the manufacture's recommendations. For calibration of equipment was used a reference strain of *Escherichia coli* ATCC 8739 according to the manufacturer's specifications. **Results:** A total of 1.310 (7%) yeasts were identified, including 434 (33.1%) *Candida albicans*, 490 (37.4%) non-albicans *Candida* and 386 (29.5%) yeasts identified at the species level. Overall, nine genera were identified. The predominant species of candida were: *C. albicans* (n=434), followed by *C. parapsilosis* (n=269), *C. tropicalis*

(n=44), *C. glabrata* (n=39), *C. guillermondii* (n=23), *C. famata* (n=22), *C. haemulonni* (n=21), *C. lusitaneae* (n=6), *C. norvegensis* (n=3), *C. catenulate*, *C. intermedia*, *C. krusei*, *C. lipolytica* and *C. pelliculosa* were identified in two samples, each and *C. dubliniensis*, *C. membranifaciens* and *C. pulcherrima* were identified in only one sample, each. 49 (3.74%) of the clinical isolates were identified only at the genus level as *Candida* spp. In the end, for other seven genus of yeast, the most prevalent were *Trichophyton* spp. (n=251), *Rhodotorula* spp., (n=53), *Tricosporon* spp., (n=30), *Cryptococcus* spp., (n=27) and *Microsporum* spp., (n=19) followed by *Geotrichum candidum* and *Hortaea werneckii* identified in one clinical sample, each. **Conclusion:** Our analysis demonstrated excellent rates for the identification of yeasts clinical isolates. Although, some limitations could be observed at the species identification level of *Candida* spp., the performance of MALDI-TOF MS technology is good for yeasts and the use of this methodology will provide direct benefits to the patient, through a more assertive empiric therapy, at a time when the rates of health care-associated infections have increased, especially by yeasts.

B-117

TranscriptomeDifferences in Normal Human Bronchial Epithelial Cells in Response to Influenza A pdmH1N1 or H7N9 Virus Infection

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Background: In 2013, a novel reassortant influenza A virus (H7N9) of avian-origin emerged in the south of China has caused 800 human infections with a mortality of 40%. Although the first epidemic has subsided, the presence of a natural reservoir and the disease severity highlight the need to evaluate its risk on human public health and to understand the possible pathogenesis mechanism. Host factors might play a critical role in the development of severe complication. Normal human bronchial epithelial (NHBE) cell cultures had been proved to be an effective model to assess the viral host interaction. In this study, we aimed to assess host differential gene expression signatures in respiratory tract epithelial cells after influenza A virus pdmH1N1 or H7N9 infection. **Methods:** The NHBE cells cultured from a 24-year-old donor were challenged by 3.0 m.o.i. pdmH1N1, H7N9, or mock control. After 12 h and 36 h incubation, the cell pellets were collected for transcriptome analysis on the GeneChip HTA 2.0 array (Affymetrix platform); the bioinformatic softwares (MetaCore™, EC1.4, TAC 3.0) were used for results evaluation. All results were duplicated. **Results:** Results of principal components analysis showed that there were significant different transcriptome profiling patterns between pdmH1N1 and H7N9 at 12 h and 36 h post infection. Totally 44699 transcripts can be detected on HTA chip, compared with mock control, absolute fold change > 2.0 (FDR < 0.05) were evaluated. At 12 h post infection, 1937 (4.33%) transcripts in pdmH1N1 infected NHBE cells and 5325 (11.91%) transcripts in H7N9 infected cells significantly differentially expressed. At 36 h post infection, differential expression of transcripts in pdmH1N1 infected NHBE cells decreased (394 [0.88%]) whereas differentially expressed transcripts in H7N9 infected NHBE cells increased (6469 [14.47%]). Gene Ontology enrichment analysis revealed that the cellular repair related pathway which includes cytoskeleton remodeling pathway and keratin filaments pathway were significantly inhibited (keratin 4 gene expression fold change -640) in the H7N9 infected NHBE cells. However, the immune regulation related gene expression significantly increased in H7N9 infected group. **Conclusion:** Gene expression pattern in pdmH1N1-infected NHBE cells is significantly different from that in H7N9-infected NHBE cells. H7N9 virus infection induces stronger immune responses but damage cellular repair mechanisms at the same time. Our study results provide valuable insights to virus-host interactions between H7N9 and NHBE cells, which also help us having more understandings on the pathogenic mechanisms that lead to severe complications.

B-118

Risk of HCV RNA Contamination by the cobas® e 602 Serology Module Prior to Nucleic Acid Testing by the cobas® HCV Test

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Background: Diagnosis of Hepatitis C involves antibody screening and confirmation of current infection by use of an HCV RNA nucleic acid test (NAT). Due to the potential risk for HCV RNA cross-contamination on serology devices employing a fixed needle for sample transfer, most laboratories either require a second blood draw from patients or a pre-serology aliquot from the primary serum specimen (in anticipation of a positive screen) in order to confirm active infection. As such constraints might jeopardize patient follow-up rates or place additional workflow burden on the lab, the ability to streamline the process and to allow the single specimen vial use for both testing procedures is of high importance. Here, we sought to assess the potential risk of HCV RNA cross-contamination by a serology screening instrument that employs disposable tips for sample transfer as an up-front process step to NAT confirmatory testing. **Methods:** Positive plasma specimens were generated by diluting armored HCV RNA (Roche Molecular Systems, Pleasanton, CA) at 6 Log and 7 Log IU/mL into normal human plasma (SeraCare Life Sciences, Millford, MA) to mimic high-positive clinical titers. Negative (n=60) and positive (n=60) plasma specimens were loaded onto the **cobas e** 602 module of the **cobas**® 8000 system in an alternating fashion and tested with the Elecsys® Anti-HCV II assay (Roche Diagnostics, Mannheim, DE); fresh negative specimens were loaded for an additional run, for a total of 120 negative cases. The HCV RNA-negative plasma samples were tested with the **cobas** HCV test for use with the **cobas**® 6800/8800 systems (Roche Molecular Systems, Branchburg, NJ) with a LoD of 8.5 IU/mL, to assess contamination potential of serology processing.**Results:** Testing of HCV RNA-negative plasma samples for the presence of low-level HCV RNA resulted in no detectable positive signal and an overall serology processing cross-contamination rate of 0% (95% Confidence Interval 0.00 - 0.03) (0/120). **Conclusion:** Hepatitis C antibody reactive specimens analyzed on the **cobas e** 602 serology module may be suitable for direct, primary specimen reflex testing by a sensitive HCV RNA confirmatory test, but additional studies are warranted. While this study design aimed to challenge the potential for contamination during serology processing by alternating high-positive and negative specimens, it does not mimic typical clinical laboratory presentation, which on average includes lower vial titers in a more randomized pattern. Nevertheless, the results herein demonstrate no risk of HCV RNA cross-contamination and that automated processes that minimize the need for manual intervention during the transfer of specimens, either prior to or after **cobas e** 602 assessment, may further reduce the chance of a contamination event.

B-119

Monitoring the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children: protective anti-HBs levels and cellular immune responses

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Vaccination against hepatitis B virus (HBV) is recommended worldwide. The aim of this study was to assess the efficacy of infant hepatitis B vaccination and revaccination in 0- to 8-year-old children in the context of protective anti-HBs levels and cellular immune responses. Using a random questionnaire survey, 1,695 pre-school children were recruited as research subjects during January 2015 to June 2017. Blood samples were obtained to measure HBV serological markers as well as peripheral immunocytes. The children were divided into non-, low- and hyper- responsive groups (NR, LR, and HR) based on the vaccination efficacy. Additionally, the effect of revaccination on the NR group was evaluated at 1 month after completion of the vaccination course. Among a total of 1,695 children, 1,591 (93.86%) were infants who were followed while undergoing their primary course of hepatitis B vaccination at the 0-1-6 month schedule, and 1,249 (79.30%) of them developed antibodies against HBsAg (anti-HBs) titers greater than 10 IU/L. The results of immunocyte studies indicated that the $CD8^+$ T cells, $CD4^+CD45RO^+$ T cells, $CD8^+CD45RA^+$ T cells, and T follicular helper (Tfh) cells increased significantly in NR compared with HR. However, lymphocytes, $CD4^+$ T cells, and $CD4^+CD45RA^+$ T cells in NR were lower than that in HR. 96 of the non-response cases showed seroprotection after revaccination among 103 cases. Therefore, most of the preschool children who received hepatitis B vaccine in infancy achieved significant seroprotection. Seroconversion rates of individuals revaccinated after initial vaccination failure were significantly higher than those after primary vaccination. Different vaccination efficacy groups showed significant changes in circulating immunocytes, which might be a factor affecting the recombinant HBV vaccine's immune effectiveness.

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B-120

Diagnostic performance of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

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Background: Xpert MTB/RIF assay (Xpert) has the potential to accurately diagnose pulmonary tuberculosis in high and low burden countries. The purpose of this study was to evaluate the diagnostic performance of Xpert during routine clinical use in an intermediate burden setting. **Methods:** Between July 2014 and December 2016, a total of 2,952 consecutive respiratory specimens were simultaneously tested by Xpert, mycobacterial culture, and smear microscopy. **Results:** Compared with mycobacterial culture as the reference, the overall sensitivity, specificity, PPV, and NPV of Xpert were 74.1%, 97.5%, 74.7%, and 97.5%, whereas those of smear microscopy were 38.8%, 96.7%, 53.1%, and 94.2%, respectively. The sensitivity of Xpert was higher among smear-positive specimens compared with smear-negative specimens (96.1% [90.3-98.9] versus 60.2% [52.3-67.9], $P < 0.0001$), whereas the specificity of Xpert was lower among smear-positive specimens compared with smear-negative specimens (92.2% [84.6-96.8] versus 97.7% [97.1-98.3], $P < 0.01$). The sensitivity of smear microscopy was higher in early morning sputa compared with spot sputa (76.9% versus 35.2%, $P < 0.01$) and its specificity was higher in inpatients compared to outpatients (97.8% versus 94.7%, $P < 0.0001$). However, the diagnostic performance of Xpert was not affected by those factors of heterogeneity. **Conclusion:** Our data showed that performance of Xpert assay was more stable and superior to smear microscopy for diagnosis of pulmonary tuberculosis during routine clinical use in an intermediate tuberculosis burden setting.

B-121

Quantitative capabilities of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

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Background: Xpert MTB/RIF assay (Xpert) has the potential to predict smear microscopy grade in high and low burden countries. The purpose of this study was to evaluate quantitative capabilities of Xpert for prediction of smear microscopy grade during routine clinical use in an intermediate burden setting. **Methods:** Between July 2014 and December 2016, a total of 2,952 consecutive respiratory specimens were simultaneously tested by Xpert, mycobacterial culture, and smear microscopy. **Results:** Among a total of 110 smear-positive specimens, 104 were Xpert-positive, corresponding to the overall sensitivity of 94.5%, whereas among a total of 2,576 smear-negative specimens, 2,422 were Xpert-negative, corresponding to the overall specificity of 94.0%. Among a total of 258 Xpert-positive specimens, 104 were smear-positive: Xpert semiquantitative results categorized as high, medium, low, and very low predicted 100% (15/15), 79.1% (53/67), 28.8% (30/104), and 8.3% (6/72) of smear-positive specimens, respectively, whereas Xpert predicted 99.8% (2,422/2,428) of smear-negative specimens. The semiquantitative result of Xpert had a strong correlation with smear microscopy grade for mycobacterial burden prediction (Goodman-Kruskal $\gamma = 0.982$, $P < 0.0001$). Among a total of 154 Xpert false-positive patients, 37 (24.0%) had cavitations on chest radiological findings, indicating high transmission potential of suspected pulmonary tuberculosis patients with Xpert false-positive results based on the initial negative smear examination. However, of 6 Xpert false-negative patients based on the initial positive smear examination, 1 (16.7%) presented pulmonary cavity, which suggested that Xpert-negative results could not perfectly rule out non-infectiousness of suspected pulmonary tuberculosis patients. **Conclusion:** Xpert semiquantitative results can provide a novel standardized strategy to measuring bacillary load in the sputum of patients with pulmonary tuberculosis.

B-122

Detection and quantification of Hepatitis C Virus using the new Aptima HCV Quant Dx assay in the fully automated Panther® System compared to the Abbott Realtime HCV assay.

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Background: Hepatitis C Virus (HCV) continues to be an important health concern worldwide. Different therapeutic methods are now available for the treatment of HCV infection with good results. Determining the viral load of patients under treatment is now the standard of care for monitoring the response to these treatments. There are different commercially available assays used to detect and quantify HCV RNA in serum and plasma specimens. The objective of this study was to compare the Aptima HCV Quant Dx assay, recently released by Hologic, Inc®, with the Abbott Molecular Realtime HCV assay. The Aptima HCV Quant Dx assay is a real-time transcription-mediated amplification (TMA) test, run in the Panther System (Hologic) used for confirmation of diagnosis and monitoring of HCV RNA. The Abbott Realtime HCV assay is an RT-PCR test run on the automated m2000 system (Abbott Diagnostics). **Methods**: Sixty plasma specimens, twenty negatives and forty positives for HCV were included in this study. All sixty specimens were used to test the qualitative performance and thirty of them, with known viral loads, were used to test the quantitative performance. All these specimens had been previously tested on the Abbott m2000 platform. The specimens were assayed using the Aptima HCV Quant Dx Assay on the Panther System following the manufacturer instructions. Specificity of the new assay was tested using 20 HCV negative specimens, some of which were positive for Cytomegalovirus (CMV) and Human Immunodeficiency Virus (HIV). Precision was tested using a known HCV positive specimen repeated twelve times in different runs. Results obtained from specimens tested in both instruments were compared using the EP Evaluator program. **Results**: The EP Evaluator software was used to determine whether the methods are equivalent within a total allowable error of 1 log_{10} IU/mL. Thirty specimens with known HCV genotypes 1a, 1b, 2b, and 3a were compared over a range of 1.11 to 6.98 log_{10} IU/mL. The test passed with 98.3 % agreement. One specimen with low viral load was negative on the Abbott instrument and positive on the Panther system. This could be explained because the Aptima HCV Quant Dx Assay has a lower detection limit (<3.9 IU/mL) than the Abbott System (<12 IU/mL) in plasma specimens. The difference between the two methods was within allowable error. The average error index was 0.12 with a range of -0.41 and 0.54. The coefficient of correlation (R) between both methods was 0.9951. For the precision study, the EP Evaluator results showed a mean of 3.861 log_{10} IU/mL with a standard deviation of 0.047. This value was within the 2 SD range (3.767-3.954). **Conclusions:** We can conclude that the Aptima HCV Quant Dx assay is a highly sensitive, accurate, and reproducible assay with a performance equal to that of the Abbott Realtime HCV assay. The Aptima HCV Quant Dx assay is a faster and more efficient test than the latter. This is helpful in the lab setting because it reduces hands on time needed to set up the test and allows for shorter wait time for results.

B-123

Method comparison of the VITROS ® Immunodiagnostic Products Anti-*T. cruzi* (Chagas) Assay* to the Ortho® *T. cruzi* ELISA Test System

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Background: This study was designed to compare the clinical performance of the VITROS Immunodiagnostic Products Anti-*T. cruzi* (Chagas) assay (VITROS Anti-*T. cruzi* assay)* to the FDA licensed and CE-marked Ortho *T. cruzi* ELISA Test System (Ortho *T. cruzi* ELISA). **Methods:** All testing in this study on the VITROS Anti-*T. cruzi* assay* was split across the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated Systems. The Ortho *T. cruzi* ELISA testing was performed on the Verseia Integrated Processor. The clinical samples included 5210 human serum and plasma samples, including 200 presumed negative hospitalized patient samples and 5010 low risk blood donor samples, 418 presumed *T. cruzi* serological positive samples and 63 samples from subjects characterized as parasite positive by historical identification of *T. cruzi* parasites. The presumed serological positive samples were determined to be reactive on at least two other serological methods prior to this study. **Results:** For the 5010 low risk blood donor samples there was 100% agreement between methods with all samples being non-reactive with both methods. For the 200 hospital-

ized patient samples there was also 100% agreement between methods with all samples being non-reactive with both methods. For the 63 parasite positive samples there was 100% agreement between methods with all samples being reactive on both methods. For the 418 presumed serological positive samples there was 100% agreement between methods with all 418 samples being reactive on both methods. This resulted in an overall agreement of 100% for these 5691 reactive and non-reactive clinical samples. **Conclusion:** The VITROS Anti-*T. cruzi* (Chagas) assay* demonstrated equivalent clinical performance in the detection of *T. cruzi* antibodies to the FDA licensed and CE-marked Ortho *T. cruzi* ELISA Test System.*Under development.

B-124

An Evaluation of Performance of the VITROS ® Immunodiagnostic Products Anti-*T. cruzi* (Chagas) Assay*

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Background: This study was designed to assess the clinical and analytical performance of the VITROS Immunodiagnostic Products Anti-*T. cruzi* (Chagas) assay (VITROS Anti-*T. cruzi* assay)* on the VITROS ECi/ECiQ Immunodiagnostic Systems, the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated Systems. The assay detects human antibodies to *Trypanosoma cruzi*, the causative agent of Chagas' disease. **Methods:** Antibody detection in the VITROS Anti-*T. cruzi* assay* is achieved using lysate antigens coated onto the well. Sample is added to the coated wells in the first stage of the reaction, and *T. cruzi* antibody from the sample is captured. After washing, HRP conjugated murine monoclonal anti-human IgG antibodies are added. Following a final wash, bound HRP conjugates are detected using the VITROS signal reagent. Specificity was assessed using 5210 human serum and plasma samples, including 200 presumed negative hospitalized patient samples and 5010 blood donor samples. Sensitivity was evaluated using 63 samples from subjects characterized as parasite positive by historical identification of *T. cruzi* parasites. Seroconversion sensitivity was assessed by testing a commercially available panel. Assay reproducibility was assessed using two reagent lots with a 5 member panel. Analytical sensitivity was determined by testing serial dilutions of the WHO 1st International Standard (*T. cruzi* I and II) for Chagas in three determinations across two reagent lots. **Results:** The specificity of the VITROS Anti-*T. cruzi* assay* for the combined blood donor and hospitalized patient populations was 100.0% (5210/5210) [95% exact CI (99.93-100.00%)]. The sensitivity for parasite positive samples was 100.0% (63/63) [exact 95% CI (99.3-100.0%)]. For the seroconversion panel all seropositive bleeds were reactive. For the reproducibility study the observed precision for the 4 reactive panel members ranged from 2.8 to 9.0 %CV. The overall sensitivity for the WHO Chagas (anti-*Trypanosoma cruzi* I) antibody standard (09/188) was a mean of 31.2 mIU/mL (range 27.2 to 35.5 mIU/mL) with a calculated endpoint titer mean of 32.4 (range 28.2 to 36.8). The overall sensitivity for the WHO Chagas (anti-*Trypanosoma cruzi* II) antibody standard (09/186) was a mean of 59.5 mIU/mL (range 54.4 to 63.6 mIU/mL) with a calculated endpoint titer mean of 32.4 (range 28.2 to 36.8). **Conclusion:** The VITROS Anti-*T. cruzi* Assay* demonstrates excellent clinical and analytical performance in the detection of human *T. cruzi* antibodies. *Under development

B-125

Novel ELISA based on antigens from Strongyloides papillosus instead of Strongyloides ratti exhibits increased serological specificity

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Background: Strongyloidiasis is an infectious disease caused by the nematode Strongyloides. Human infection by Strongyloides stercoralis can manifest with dermatological, intestinal and pulmonal symptoms frequently passing into a chronic disease. Low parasitic loads and discontinuous larvae excretion may hamper diagnosis by coproscopy. Serological test systems are more sensitive to detect the infection. Available serological tests are commonly based on native antigens from S. ratti larvae and lack specificity. We developed and evaluated the first ELISA based on S. papillosus to increase specificity. **Methods:** Evaluation of the ELISA based on S. papillosus was performed using the following three approaches: [1] Participation in an external quality assessment scheme (NEQAS, UK) encompassing six positive and five negative samples [2] A correlation study with the commercial Bordier ELISA (Strongyloides ELISA kit based on S. ratti antigens; Bordier Affinity Products, Switzerland) including 89 sera precharacterized as either positive (n = 59) or negative (n = 30) by means of Bordier ELISA [3] Comparison with an in house ELISA based on S. ratti by determining specificity with respect to a cross-reactivity panel (n = 193, samples from patients with other parasitic or bacterial infections) and a control panel (n = 688, samples from 500 healthy blood donors, 100 pregnant women and 88 children) **Results:** [1] Results obtained with the Anti-Strongyloides ELISA were 100 % in agreement with NEQAS target values. [2] In 74 of 89 samples (83,1%), the result of the novel ELISA correlated with the Bordier ELISA. Seven discrepant cases, which were positive in Bordier ELISA but negative in the novel ELISA, were further examined. Serological analyses indicated the presence of antibodies against other parasites (Plasmodium spp., Schistosoma spp. and Echinococcus spp.) in six of these cases. [3] The S. ratti based ELISA was reactive in 13,9% of the sera in the cross-reactivity panel and in 10,6% of the samples from healthy individuals, yielding a combined specificity of 88,6 %. In comparison, reactivities of 6,2% (cross-reactivity panel) and 3,5% (healthy individuals) were detected with the novel Anti-Strongyloides ELISA, resulting in a combined specificity of 95,9%. **Conclusion:** The novel Anti-Strongyloides ELISA reveals a high diagnostic accuracy in the serological diagnosis of Strongyloidiasis. The use of native antigens from S. papillosus instead of S. ratti increases assay specificity by 7,3%.

B-126

Recombinant antigens improve sensitivity and allow species differentiation in echinococcosis diagnostics

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Background: Cystic and alveolar echinococcosis (CE and AE) are caused by the tapeworms Echinococcus granulosus and Echinococcus multilocularis, respectively. Serological tests should be used before invasive methods according to CDC guidelines. For species differentiation, blot techniques using specific recombinant antigens are of increasing significance. **Methods:** We tested 329 clinically and serologically (ELISA or Western blot) precharacterized sera. Among these were 55 CE and 52 AE samples, 122 samples of patients with other parasitic infections (including the following species: Fasciola hepatica, Strongyloides stercoralis, Taenia solium, Trichinella spiralis, Schistosoma ssp., Plasmodium ssp., Toxocara spp., Entamoeba histolytica, Leishmania ssp., Ascaris lumbricoides, Anisakis simplex and Filarioidea types), 50 healthy blood donors and 50 tumor patients. Anti-Echinococcus species-specific IgG was determined using a Western blot with electrophoretically separated Echinococcus multilocularis metacestode vesicle fluid (EmVF) and 3 membrane chips coated with recombinant E. granulosus antigen EgAgB and E. multilocularis antigens Em18 and Em95. Bands were automatically evaluated using a commercial software (EUROLineScan, Euroimmun). **Results:** Testing the pre-characterized patient sera, the conventional Western blot achieved a sensitivity of 89% at a specificity of 100% for echinococcosis, and with added recombinant proteins an increased sensitivity of 93% (at 100% specificity). Since the evaluation is challenging, a specific algorithm for species differentiation was designed in the EUROLineScan software on the basis of the antibody findings. In patients positive for specific anti-Echinococcus ssp. antibodies, the causative species was correctly assigned by the software as E. multilocularis or E. granulosus in 33 of 45 AE patients and 45 of 52 CE patients, respectively. The recombinant antigens showed no cross-reactivity, while, in the Western blot, positive results were obtained in 6 out of 122 samples and only in Anisakis and Ascaris cases. **Conclusion:** Supplementing the EmVF Western blot with immobilized recombinant antigens (Anti-Echinococcus EUROLINE-WB) increases the sensitivity for echinococcosis, at a constantly high specificity, and enables differentiation between Echinococcus species. Furthermore, no cross-reactivity to diagnostically highly relevant Taenia solium, Schistosoma ssp. and Entamoeba histolytica was observed.

B-127

Improved Sensitivity for Detection of Urinary Tract Infections Using Novel Light Scattering Methodology

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Background: Urinary tract infection (UTI) is common, and urine culture is one of the highest volume tests performed in clinical microbiology laboratories. Overuse of culture can not only monopolize laboratory resources, but also lead to unnecessary antimicrobial exposure as patients may receive treatment while await-

ing culture results, putting them at risk for *Clostridium difficile* infection and adverse side effects of medications as well as promoting bacterial antimicrobial resistance. A common approach to decrease unnecessary urine culture is to screen samples using urinalysis (UA) parameters to determine those that should proceed to culture (reflex), though guidelines to optimize sensitivity for UTI detection and specificity to eliminate unnecessary culture have not been defined. The objective of this study is to compare a novel UTI detection method (BacterioScan 216Dx UTI System) to urinalysis for screening urine samples for reflex to culture. **Methods:** Urine samples (n=124) submitted for culture were evaluated by urinalysis and a novel laser light scattering device (216Dx) used to detect the presence/absence of UTI pathogens in urine after dilution in Tryptic Soy Broth (TSB) and 190 minutes of optical assessment. Reflex parameters for culture as defined in our institution were compared to results from 216Dx to evaluate sensitivity and specificity for UTI detection defined as growth in culture of one or two uropathogens at concentrations of ≥10,000 CFU/ml. **Results:** 124 urine samples were evaluated by UA, culture and 216Dx. The 216Dx demonstrated a 100% sensitivity and 82.24% specificity for the detection of UTI, compared to UA at a sensitivity of 88.24% and a specificity of 71.96%. **Conclusion:** Screening tests are optimized for sensitivity to decrease the risk of false negative results that may harm patients. However, as specificity decreases more samples must proceed to confirmatory testing. In this study, UA demonstrated a sub-optimal sensitivity and specificity with 2 false negatives and 30 false positives. The use of a novel screening method to evaluate whether urine samples should proceed to culture provided optimal sensitivity (100%) and an improved specificity leading to 0 false negatives and fewer false positives (19 vs 30). This screening approach could lead to improved antimicrobial stewardship and patient care through fewer patients receiving antimicrobial agents while awaiting urine culture results.

B-128

Genetic polymorphisms of long non-coding RNA *RP11-37B2.1* associate with susceptibility of tuberculosis and adverse events of anti-tuberculosis drugs in the Western Chinese Han population

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Background: Little knowledge about the biological functions of *RP11-37B2.1*, a newly defined long non-coding RNA (lncRNA) molecule, is currently available. Previous studies have showed that rs160441, located in the *RP11-37B2.1* gene, is significantly associated with tuberculosis (TB) both in a Ghanaian and the Gambian populations. **Methods:** We investigated the influence of several SNPs within lncRNA *RP11-37B2.1* on the risk and manifestations of TB and the possible correlation with adverse drug reactions (ADRs) from TB treatment in a Western Chinese population. Five SNPs within lncRNA *RP11-37B2.1* were genotyped in 554 TB patients and 561 healthy subjects using the improved multiplex ligation detection reaction (iMLDR) method, and the patients were followed up monthly to monitor the development of ADRs. **Results:** No significant association between the SNPs of lncRNA *RP11-37B2.1* and TB susceptibility was observed in total samples (all p values > 0.05). Surprisingly, significant associations were observed between rs160441, rs218916 and rs218936 and thrombocytopenia development during anti-TB therapy under the dominant model (rs160441: CC: 2.42% vs. CT + TT: 7.32%; rs218916: CC: 1.46% vs. CT + TT: 7.29%; rs218936: CC: 2.25% vs. CT + TT: 6.92%, respectively) with the estimated $p = 0.014$ [odds ratio (OR) = 3.18], 0.003 (OR = 5.32) and 0.018 (OR = 3.23), respectively. **Conclusion:** Our findings firstly exhibit that three lncRNA *RP11-37B2.1* SNPs significantly associate with the occurrence of thrombocytopenia and suggest *RP11-37B2.1* genetic variants may potentially act as the useful biosignatures for identifying TB patients at greater risk of thrombocytopenia development during anti-TB treatment.

B-129

Evaluation of several FDA-cleared *Borrelia burgdorferi* ELISAs within modified two-tiered testing algorithms

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Background: Serology testing for *Borrelia burgdorferi* infection consists of a two-stage algorithm, referred to as standard two-tiered testing (STTT). In the STTT algorithm, specimens are initially assayed by methodologies such as IFA or ELISA, and equivocal or positive specimens are subsequently tested via immunoblotting for detection of IgG and/or IgM antibodies. While specific, the immunoblotting portion of the STTT algorithm contains drawbacks such as insensitivity for detecting acute infection, subjectivity of result interpretation, and technically challenging procedures. Consequently, there are published studies that support the replacement of immunoblotting with a more sensitive and automatable methodology such as ELISA; referred to as modified two-tiered testing (MTTT). The objective of this study was to evaluate the performance of several FDA-cleared *Borrelia burgdorferi* ELISAs within MTTT algorithms. **Methods:** 280 clinically-characterized and blind-coded serum samples (termed Premarketing Panel), were obtained from the Centers for Disease Control and Prevention (CDC). The Premarketing Panel was initially tested by the *Borrelia burgdorferi* IgG/IgM ELISA Test System (ZEUS Scientific, part# 3Z9651), as well as the VlsE1/pepC10 IgG/IgM ELISA Test System (ZEUS Scientific, part# 3Z9661), and the raw data were submitted to the CDC for decoding and assembly relative to preexisting clinical and STTT data (VIDAS®/Marblot™). The Premarketing Panel was subsequently tested by the *Borrelia burgdorferi* IgG ELISA Test System (ZEUS Scientific, part# 3Z9651G), *Borrelia burgdorferi* IgM ELISA Test System (ZEUS Scientific, part# 3Z9651M), and the C6 Lyme ELISA™ (Immunetics, cat# DK-E352-096). The ELISA data derived from testing the Premarketing Panel were assembled and analyzed according to the following six MTTT algorithms: [1. 1st Tier - *Borrelia burgdorferi* IgG/IgM ELISA, 2nd Tier - VlsE1/pepC10 IgG/IgM ELISA], [2. 1st Tier - *Borrelia burgdorferi* IgG/IgM ELISA, 2nd Tier - C6 ELISA], [3. 1st Tier - *Borrelia burgdorferi* IgG and/or IgM ELISA composite result, 2nd Tier - VlsE1/pepC10 IgG/IgM ELISA], [4. 1st Tier - *Borrelia burgdorferi* IgG and/or IgM ELISA composite result, 2nd Tier - C6 ELISA], [5. 1st Tier – VIDAS IgG/IgM, 2nd Tier - VlsE1/pepC10 IgG/IgM ELISA], [6. 1st Tier - VIDAS IgG/IgM, 2nd Tier - C6 ELISA]. **Results:** Of the 30 acute Lyme disease samples, the STTT algorithm detected 14 (47%), and the MTTT algorithms (numbered 1-6 above) detected 22 (73%), 20 (67%), 23 (77%), 21 (70%), 21 (70%), and 20 (67%) respectively. Of the 90 total Lyme disease samples (representing disease stages 1-3), the STTT algorithm detected 66 (73%), whereas the MTTT algorithms (numbered 1-6 above) detected 77 (86%), 75 (83%), 80 (89%), 78 (87%), 76 (84%), and 75 (83%) respectively. Of the 190 non-Lyme disease control samples, the STTT algorithm detected 0 (0%), and the MTTT algorithms (numbered 1-6 above) detected 2 (1.1%), 0 (0%), 2 (1.1%), 1 (0.5%), 3 (1.6%), and 1 (0.5%) respectively. **Conclusion:** This study represents the first MTTT evaluation of several ELISAs that are currently FDA-cleared for use as 1st tier tests. The novel data presented herein are consistent with previously published literature, and support the notion that the MTTT algorithm yields improved sensitivity for detection of early Lyme disease, while maintaining acceptable specificity.

B-130

Universal Pathogen Capture System for Rapid Isolation of Intact Bacteria Directly from a Patient Sample

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Background: Currently, patient blood samples have to be cultured prior to performing genetic, mass spectrometric, or phenotypic analyses for bacterial identification. While recent methods have reduced the time to identify the bacteria, the rate-limiting step is still blood culturing. Culture-independent methods rely on antibody or DNA-based recognition for capturing the bacteria. While in principle a large number of bacteria can be captured, in practice, the number of pathogens captured are limited to those that can be recognized by the antibody/ DNA on the chip/kit, which is a small number. Further, these methods are unable to process the entire patient sample volume which lowers their overall diagnostic accuracy. We report a new approach to isolate intact bacteria directly from patient blood samples in < 1 hour, which does not rely on antibodies or DNA primers. The system isolates intact bacteria by selectively breaking down blood cells but not bacteria, separating the intact bacteria from the lysis debris by filtration, and concentrating them for multiplex analysis. The very highly selective lysis of blood cells is accomplished by leveraging the differential response of blood cells and bacteria to mechanical forces while the filtration is performed using ultrathin defined-pore membranes. **Objective**: To evaluate the performance of this approach for isolating bacteria directly from blood. **Methods**: Bacterial strains were obtained from collaborators and ATCC and cultured prior to spiking different concentrations into 5-10 mL of whole blood containing disodium EDTA. The seeded samples were passaged through the selective lysis unit of the platform. The lysate was filtered to eliminate the lysis debris and to concentrate the bacteria. Lysis efficiency was monitored by microscopy as well as by dynamic light scattering. Bacterial recovery was measured by enumerating bacteria in the seeded sample as well as in the lysate, filtrate, and filter surface. **Results**: A total of 25 bacterial strains were analyzed that included gram positive species such as *S. aureus* and *E. faecium* and gram-negative species such as *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa*. The isolation of intact bacteria from 10mL of whole blood was completed in < 50 minutes. Recovery of intact bacteria after passage through the lysis unit was > 90% and through the full process was > 85%. Bacteria were viable after isolation. **Conclusion**: The approach offers the ability to isolate bacteria directly from whole blood in < 50 minutes without utilizing

antibodies or DNA primers. This, in turn, has the potential to both isolate a broad range of bacteria as well as to dramatically reduce the time for microbial identification by eliminating the need to culture the blood sample. The isolated bacteria can be identified by any suitable method. Further studies are needed to directly identify bacteria from clinical samples and to expand the number of pathogens analyzed. These investigations will aid in the development of a universal pathogen capture system that can help isolate and identify a broad range of bacteria directly from blood in < 1 hour.

B-131

Effects of erythropoietin on experimental Chagas disease: histopathological and cardiac biomarkers.

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Background: Chagas disease caused by the protozoan *Trypanosoma cruzi* represents a serious health public problem with high morbidity and consequent elevated mortality rate. About seven million people are infected with the parasite, mostly in Latin America. Approximately 30% of individual with chronic infection develop Chagas cardiomyopathy, the most important clinical manifestation of the disease. Chagasic cardiomyopathy presents different forms, but all of them culminate with cardiac dysfunction due to cardiomegaly, myocarditis and cardiac fibrosis. Trypanocidal drugs, despite reducing parasitaemia, have no efficacy on the progression of lesions in the chagasic heart. In this way, there is no effective treatment for these cases, condemning the patient to live with or succumb to the disease. Erythropoietin (Epo), a key-regulator of erythropoiesis, also has a cardioprotective effect by reducing the processes of apoptosis, inflammation and myocardial ischemia through the formation of new blood vessels. However, it is unknown whether the action of this protein can be effectively used both in prevention or treatment of Chagas cardiomyopathy. Thereby, this study aims to assess the possible cardioprotective effect of erythropoietin (Epo) on experimental chronic Chagas disease. **Methods:** C57BL/6 mice were randomly divided into four groups: administration of saline or Epo during 30 days before the infection (to verify the protective effect of Epo) and administration of saline or Epo during the acute phase of the disease (30dpi) (to verify the therapeutic effect of Epo). All the animals were infected by intraperitoneal route with 10^5 trypomastigotes Colombiana strain of *T. cruzi.* The activity of biomarkers of heart lesion (total creatine kinase – CK, myocardial fraction of CK – CKMB and aspartate aminotransferase – AST) were measured in blood samples before the infection (D0) and 15, 30, 90 and 180 dpi. Histopathological analysis (haematoxylin and eosin) of heart, spleen and large intestine on the chronic phase of Chagas disease (180 dpi) were performed. **Results:** A therapeutic effect of Epo was observed in CK total (p<0.001), but this did not occur in the cardiac muscle fraction of CK. In fact, the kinetics for CKMB activity throughout the *T. cruzi* infection determined in mice treated or no with Epo showed no differences. Concerning the AST activity, infected mice treated with Epo in the acute phase had increased levels in the course of the disease when compared to the D0 (p<0.001). The histopathological analysis showed lesions in evaluated tissues (heart, spleen and intestine) in all experimental groups. However, no significant difference was seemed between groups. **Conclusion:** Thus, the administration of Epo during 30 days before the *T. cruzi* infection or during the first 30 dpi does not prevent the occurrence of cardiac damage in Chagas disease.

B-132

Automated and laboratory information system integrated workflow for detection of yellow fever virus by RT-qPCR in EDTA-plasma.

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Background: In December 2016, Brazil reported the country's largest yellow fever virus (YFV) outbreak in decades. Through 16 February 2018, about 5000 suspected cases were reported, including 500 deaths. Immunologic detection methods for YFV, such as MAC-ELISA, can cross-react with others Flavivirus. Conversely, RT-qPCR can reliably and accurately detect YFV during the viremic phase (5-7 days after the symptoms-onset). RT-qPCR main drawback is its production capacity so that the development automated of tests is crucial. The present study aimed to validate an automated RT-qPCR assay for YFV detection. **Methods:** Specimens used in this validation were quality control materials positive for YFV by MAC-ELISA (Serum, n=15) donated by a Brazilian external quality assurance provider (Controllab) and EDTA-plasma samples from healthy volunteers (n=96). The control materials were tested for YFV by RT-qPCR and pooled. The pool's viral load was quantified against serial dilution of synthetic ssDNA corresponding to the YFV RT-qPCR genomic target. EDTA-plasma samples from the healthy volunteers were spiked with known amounts of the YFV to produce samples to be used in the assay validation. The RT-qPCR workflow was performed on the Flow classic solution (Roche). Nucleic acids were extracted from 500ul of the sample. An in-vitro transcribed random RNA sequence, which is not found in nature, was spiked into plasmas during the nucleic acids extraction to function as process control. Primers/probes were obtained from the literature. YFV RNA and the control RNA were assessed by multiplex RT-qPCR. The assay's limit of detection was determined by probit regression analysis of the results obtained from a viral serial dilution from ~5000 to ~0.5 copies/mL (12 replicates of each concentration). To investigate assay's precision near the LOD three samples with ~77, ~7 and ~0.7 copies/mL of the virus were evaluated using the CLSI EP12-A2 method during three days (24 replicates of each concentration). The accuracy was evaluated using a spike recovery strategy: 96 negatives and 25 positives spiked samples were prepared and tested (viral loads in positive samples ranged from 19625 to 38 copies/mL). The total, positive and negative agreements between the expected and obtained results were evaluated. Cross-reaction with Zika, dengue and chikungunya viruses was tested. **Results:** Thirteen out of 15 quality control materials positive for YFV by MAC-ELISA tested positive for YFV RT-QPCR, and their pooling yielded 196.259 viral copies/mL. The observed limit of detection for YFV RT-qPCR was 34 copies/mL (95%CI 18-103 copies/mL). Samples ~77, ~7 and ~0.7 copies/mL returned positive results in 24/24 (100%), 16/24 (66%) and 0/24 (0%) of the tested instances, respectively. The total, positive and negative agreements between expected and observed results were 100% (95%CI 96.9-100%), 100% (95%CI 86.7-100%) and 100% (95%CI 96.2-100%). Cross-reaction with Zika, dengue and chikungunya viruses were not observed. **Conclusion:** The proposed RT-PCR method for detection of YFV is highly sensitive, the assay showed the limit of detection below 50 copies/mL. Acceptable precisions were observed for positive (>77 copies/mL) and negative results (>0.7 copies/mL). The agreement between expected and observed results were complete, and the workflow can process 96 tests in 5 hours.

B-133

Identification of UTI pathogens using an open array platform on the QuantStudio 12K Flex system

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Background: Increasing multidrug resistance in uropathogens is leading to high recurrence rates for UTI's and has become a global challenge for antibiotic treatment regimens. It is extremely important to promptly and accurately identify the causative uropathogens for effective UTI management. We have custom designed an open array for rapid identification of 17 uropathogens using real time PCR technologies. The design of our urinary tract infection pathogen panel open array (UTI pathogen panel) allows testing of 48 urine samples for 17 targeted genes within five hours. DNA is extracted directly from urine samples and amplified on the Thermofisher QuantStudio 12k Flex open array system for detection of the following uropathogens; *Acinetobacter baumannii, Klebsiella pneumonia, Citrobacter freundii, Morganella morganii, Enterobacter aerogenes, Proteus mirabilis, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella oxytoca, Proteus vulgaris, Providencia stuartii, Pseudomonas aeruginosa, Staphylococcus saprophyticus, Streptococcus agalactiae* and *Candida albicans.* **Objectives:** To evaluate the UTI pathogen panel for its analytical performance characteristics and utility for patients at Accureference Medical laboratories. The real time PCR assay on the open array was compared with the currently performed traditional microbiology techniques and its performance characteristics were evaluated. **Methods:** A total of 124 urine specimens submitted for detection of UTI's by standard microbiology techniques were used for correlation studies using our UTI pathogen panel. Extraction of nucleic acids directly from urine specimens was performed using the Thermofisher MagMAX Multi sample Ultra kit on the Magmax automated extractor. Identification of uropathogens was performed on the QuantStudio 12k flex using an open array system. **Results:** Urine specimens (n=124) submitted to the microbiology laboratory for culture were tested in parallel for the presence of uropathogens using our UTI pathogen panel. A total of 90/124 specimens were identified as positive for uropathogens using the UTI panel whereas only 75 of these specimens were resulted as positive for any uropathogen by microbiology. There was 100% concordance with culture results for these 75 specimens but in 22/75 specimens (29%), at least one additional pathogen undetected by culture was identified using the UTI panel. The most frequent organisms identified in the positive specimens were *E.coli* followed by *Klebsiella pneumonia* and *Enterococcus faecalis*. Analytical sensitiv-

ity of PCR reactions for detection of the pathogens was determined by making standard curves on bacterial isolates and appropriate cut-off values were applied to correlate with bacterial loads of 10^3 colony forming units (CFU) per ml. The panel showed 100% specificity in identification of uropathogens in our studies. **Conclusions:** The UTI pathogen panel offers the advantage of identifying the cause of UTI within hours and is more sensitive than traditional microbiology methods. The panel helps to reduce the turnaround time for identification of slow growing and fastidious UTI pathogens. The molecular based semi quantitative UTI pathogen panel is a good alternative to traditional microbiology methods for sensitive and specific detection of uropathogens.

B-134

Respinning positive anti-HIV samples and retesting in duplicate - is it necessary?

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Background: Many immunoassay manufacturers recommend rerunning initial positive samples in duplicate after recentrifugation before deciding on the final result. This increases reagent usage and delays reporting. This study was designed to examine the outcome of following this practice over 6 years for the Roche Cobas Anti-HIV assay. **Methods:** Anonymised records of all repeat testing of anti-HIV analysis performed on a Roche Diagnostics e601 immunoassay analyser between 2012 and 2017 were examined in Microsoft Excel and Access. Only serum (SST II) samples are accepted for testing - all are initially spun for 4 min at 1900 g before immediate testing. Repeat testing in duplicate of initial positive samples (cutoff-index COI >=0.9) following respinning at 10 min at 4020g was performed. Any changes in result classification due to duplicate retesting was noted. **Results:** 52828 requests were received of which 98% were non-reactive. Of the remaining 960 which underwent duplicate retesting after recentrifugation, 27 were reclassified as non-reactive. The highest initial COI (iCOI) amongst these cases was 2.44 with a drop of 2.098 after respinning. The median drop in COI was 1.261. Limiting respinning and duplicate retesting to samples with iCOI 0.9-10 or 0.9-5 would reduce sample retesting by 754 and 767 cases respectively while continuing to identify the original 27 non-reactive cases. **Conclusion:** Duplicate retesting of all initially reactive anti-HIV samples is a wasteful practice. Only 2.8% of all initially reactive samples were reported as non-reactive after duplicate testing and all had low COIs. By limiting retesting to samples with COIs < 10, the number of samples undergoing retesting can be reduced by 79%.