The aim of this study is to design an ELISA for detection of the pathogen in infections show different patterns of an infectious disease.

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. 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 41-50 years and 0.6% among adolescents 11-20 years. 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 populatioin if not treated because they are care givers. Population target screening, treatment and public health enlightenment will benefit the population and reduce the socioeconomic implication of HBV infection.

Key Words: Asymptomatic Hepatitis, B Surface Antigen, Prevalence, Ibadan

Development of a Bartonella henselae specific Human IgG ELISA

M. Jost, A. Latz, V. Kempf. University Hospital, Goethe-University, Institute for Medical Microbiology and Infection Control, Frankfurt am Main, Germany; NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany; German National Consilary Laboratory for Bartonella, Frankfurt am Main, Germany.

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 in-house tests. Fraction potential fractions were further tested and optimized for ELISA.

4. Results Patients with B. henselae infections show different patterns of antigen 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 antisa 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 LOEWI III project.

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

E. O. Anto, C. Obirikorang, S. A. Sakyi, E. Achampong. Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

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-resource 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 >2+ on urine dipstick analysis. CD4 T lymphocytes count was determined using the Becton Dickinson (BD) FASCount system, CBC was analyzed 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. Receiver 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 43.09 years. Proteinuria ≥ [aOR=4.30 (3.0 to 18.5)]; leukocyturia ≥ [aOR=2.91 (1.13 to 12.5)]; hematuria ≥ [aOR=2.30 (1.08 to 9.64)]; and TLC <1200 cells/µl [aOR=5.26 (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++ (leukocyturia + 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.

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

J. Tan, M. Wong. Khoo Teck Puat Hospital, Singapore, Singapore

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. 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 procalci-
tonin, 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 procalcitonin 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 respec-
tively (p<0.0001). 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 con-
tribute 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.

T. D. OGUNLEYE, UNIVERSITY COLLEGE HOSPITAL, IBADAN, NIGERIA.

Background: Hepatitis B viral infection is a chronic infection which could lead to chron-
ic liver disease and in turn eventually leads to several other clinical outcomes associat-
ed 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 high-
est 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 labora-
tory was compiled. Hepatitis B surface antigen (HBsAg) was assayed using 
electrochemiluminescense immunoassay “ÉCLA” method on Cobas E immu-
nosays analyser, making use of sandwich test principle. Levels 1 and 2 qual-
ity 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 re-
peated for confirmation. Any result greater than 1.0 was considered reactive.

Results: A total number of 503 apparently healthy adult patients were investi-
gated. 288 (57.3%) of the population were male while 215 (42.7%) of the popula-
tion were female. A total of 108 (21.5%) of the population tested posi-
tive 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

R. A. Pinto1, W. O. Silva2. 1Patologia Clinica São Marcos, Belo Horizonte, Brazil, 2Patologia Clinica São Marcos, Belo Horizonte, Brazil

Background: Since the 80s, Brazil has instituted as state politic, a Public Healthcare 
System that, in essence, values the universal, equal and integral access. 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 - amongst them, the STI/AIDS and Viral Hepatitis from the Ministry 
of Health. In this aspect, even with educational campaigns, and the access 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 determines the use of, at least, one of the Six Flow Chart. In our Lab,
Wednesday, August 1, 9:30 am – 5:00 pm

Infectious Disease

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.


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 viremia 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 personalized 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 TaqMan 48 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 Log10 7.22 IU/mL, were tested on the Cobas 4800 system. Precision studies were derived from quality control materials at HCV viral loads of Log10 2.38 and Log10 6.36 IU/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 0.9722 ± 0.0085 (R=0.9967). A (-) Log10 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; Log10 2.38IU/mL) and 0.05 (HCV; Log10 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 Taq-Man48 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

K. Hoekstra. Quest Diagnostics, PeaceHealth United General Medical Center, Sedro-Woolley, WA

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-field side 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 an-gina resolved. Fourteen days after onset of the illness, the patient’s hematocrit and troponin were within normal reference range, the targe 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 antifungal but not antifun-gal 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

E. Ito1, S. Yamakado1, N. Kawada1, A. Ono1, K. Nakaiishi2, S. Watabe2.
1Waseda University, Tokyo, Japan, 2TAUNS Laboratories, Inc., Shizuoka, Japan

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 μl 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 MPB64, 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 (3e-hydroxyestroid dehydrogenase) with co-factors (NADH and thio-NAD) and substrates (androstereon 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 Mycobacterium 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 × 10^6 copies/μl (i.e., 4.2 × 10^6 copies/μL), and that the limit of determination was 1.3 × 10^6 copies/μl (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/μl (i.e., 1.4 × 10^4 copies/μL), and the limit of determination was 4.2 × 10^6 copies/μl (i.e., 4.7 × 10^4 copies/μL). Using the plasmid, the limit of detection was 7.0 × 10^6 copies/μl (i.e., 7.8 × 10^6 copies/μL), and the limit of determination was 2.3 × 10^6 copies/μl (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 result 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**

D. A. G. Zauli, F. Cueva Mateo, Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil

**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-virus, 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 triplicates with concentration of RNA in the range of 1.250 to 19 copies/µL); (iii) In-trassay 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 (r²) of 0.99 and amplification efficiency of 94% (NCR) and 103% (ENV). The experiments performed to evaluate the precision demonstrated optimal reproducibility 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?**

F. L. O. Marinho, F. Cueva Mateo, D. A. G. Zauli. Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil

**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 Yellow 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 Northwest. In Southeast region, 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 HPI 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**

E. Machetti-Mareca1, I. Valledor2, M. Gil-Rodríguez3, F. J. Castillo4, C. Seral5, D. R. Herrero6, 1Facultad de Ciencias, Universidad de Zaragoza. 2Centro Biotec S.L., Zaragoza, Spain. 3Facultad de Medicina, Universidad de Zaragoza. 4Centro Biotec S.L., Zaragoza, Spain. 5Hospital Clínico Universitario Lozano Blesa, Facultad de medicina, Universidad de Zaragoza. 6IIS Aragón, Departamento de Microbiología, Zaragoza, Spain, 7Centro Biotec S.L., San Mateo de Gállego, Spain

**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, VIA-SURE RNA-DNA Extraction Kit (Certeq Biotech, Spain). Two quick processes were compared to RS, VIASURE Lysis Buffer (LB) which has been recently developed by Certeq, 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 Diagnostics) using VIASURE gastrointestinal panel (Certeq). **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 not 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 GI, MS was isolated with three different types of extraction procedures. The reference standard (RS) was a commercially available kit using a silica-based matrix, VIA-SURE RNA-DNA Extraction Kit (Certeq Biotech, Spain). Two quick processes were compared to RS, VIASURE Lysis Buffer (LB) which has been recently developed by Certeq, 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 Diagnostics) using VIASURE gastrointestinal panel (Certeq). **Conclusions:** For viruses, in almost all cases were considerably greater than 3. In Astrovirus and Norovirus GI, MS was isolated with three different types of extraction procedures. The reference standard (RS) was a commercially available kit using a silica-based matrix, VIA-SURE RNA-DNA Extraction Kit (Certeq Biotech, Spain). Two quick processes were compared to RS, VIASURE Lysis Buffer (LB) which has been recently developed by Certeq, 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 Diagnostics) using VIASURE gastrointestinal panel (Certeq).
Comparison of HDV RNA level and severity of liver disease among subjects with HDV/HBV infection.

U. Gambi1, O. Chibati1, T. Boldoo2, T. Enkh-Amgalan1, N. Nymadawa1, G. Ferri, L. Bavagnoli, L. Spinelli.

1Gyals Medical Center, Ulaanbaatar, Mongolia, 2National Center for Communicable Diseases of Mongolia, Ulaanbaatar, Mongolia, 3Regional Diagnostic Center, Dornod Province, Mongolia

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) and their liver damage status.

Methods: We conducted a retrospective study to record results for 46 subjects (age 26-72, female 22, male 24) who were tested positive for both HDV RNA (GeneSis, UK) and HBV DNA (Abbotti, 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 the subjects’ alanine aminotransferase (ALT) levels and their liver damage status (ASC/asymptomatic, CH-chronic hepatitis, LC-liver cirrhosis) diagnosed by doctors.

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.

Relationship between level of HDV RNA (copies/ml) and of HBV DNA (IU/ml) in serum of liver cirrhosis (LC) subjects.

B-061

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


Middle Eastern 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 POCKET™ COMBO system (GeneReach), including a compact automatic nucleic acid extraction device (taco™ mini) and a simple insulated isothermal PCR device (POCKET™ Mini), enables pathogen detection at settings close to points of need. Clinical performance of a qualitative matrix MERS-CoV RT-PCR method targeting both sgrepe and ORF1a marker genes (LoD95%, 30 and 17 genome equivalents, respectively) on the POCKET™ system were evaluated. Clinical performance of the index matrix RT-PCR and the reference RT-qPCR 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 (Qagen) 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.

Results: 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. Two of three and three were reference RT-qPCR positive/RT-qPCR negative. Interrater agreement calculated by kappa test was 96.08% (C195%, 91.62 ~ 100%; k = 0.92), indicating the index matrix RT-PCR and the reference RT-qPCR had excellent agreement. Components of the POCKET™ Combo system have also been integrated into an automated sample-to-results device to help reduce hands-on time and enhance test consistency.

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.

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


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 lophylized 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.
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 23 follow-up samples covering 124 days from a male was analysed. In addition two patients from a reference laboratory (Lubeck) 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 higher 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.

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**B-064**

A brazilian case report of yellow fever infection

F. L. O. Marinho, E. Cueva Mateo, D. A. G. Zauli, Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil

**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 Instituto 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. Conclusion: 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.

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**B-065**

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

V. Murthy, V. MacKean, J. Leach, C. Huang, R. Vemula, J. Wu, B. Anake-Ila. SeraCare Life Sciences, Milford, MA

**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
**Biochemistry**

**B-066**

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

A. J. Mena Lora1, P. Gonzalez2, M. Lubero2, B. Billini2, G. Grau1, S. C. Blesadable1, University of Illinois at Chicago, Chicago, IL, 2Laboratorios Amadita, Santo Domingo, Dominican Republic, 3Laboratorios Amadita, Chicago, Dominican Republic

**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 (Vitek2®/bioMérieux) 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 (VRS) 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 (%)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MRSA</th>
<th>MSSA</th>
<th>MRSA/MSSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>11.7</td>
<td>13.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>31.8</td>
<td>31.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2.8</td>
<td>3.1</td>
<td>1.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>3.9</td>
<td>3.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Lincomycin</td>
<td>8.3</td>
<td>8.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Methicillin</td>
<td>43.5</td>
<td>43.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>5.3</td>
<td>5.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**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.

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**B-067**

**Evaluation of a Procalcitonin Assay on the Atellica IM Analyzer**

A. V. Rybin, J. Freeman. Siemens Healthineers, Tarrytown, NY

**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 proteolytic cleavage 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**

**B-068**

**Optimization of the lamination system of urine samples in Flow Cytometry**

P. M. B. S. Junior1, V. B. Santos1, L. H. Souza1, M. E. F. Reis1, D. M. V. Gomes2, S. V. L. Argolo1, G. A. Campana2, 1DASA, Duque de Caxias, Brazil, 2DASA, São Paulo, Brazil

**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 6,500 gallons of 0.7 day, reaching 231.8 gallons / year. And an annual financial gain of $6,500.
Real Time PCR Detection
Wednesday, August 1, 9:30 am – 5:00 pm

B-070
Ready-to-use Stabilized qPCR Assays for Detection of Zika, Dengue and Chikungunya Viruses

M. E. Teresa-Rodríguez1, C. Escobar1, S. Fernández-Laguarda1, D. R. Hernandez1, F. Yu1, S. Zheng1, X. Li1, J. A. Noguera-Velasco2, A. Martínez-López de Castro2.
1First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China
2Facultad de Veterinaria, Universidad de Zaragoza, Certest Bioteo S.L., Zaragoza, Spain

Background:
Zika virus (ZIKV), Dengue virus (DENV) and Chikungunya virus (CHIKV) are arthropod-borne arboviruses transmitted by mosquitoes of the Ae. 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 as 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 Assessment (EQA) programs QCMD and INSTAND. Samples were collected from April 2014 to October 2017. Genomic RNA was isolated using "QIAMP 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 Bioteo), 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

F. Yu, S. Zheng, X. Li, Y. Chen.
First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China

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 two influenza virus in screening influenza A and B were selected, including three common subtypes of sH3N2, H1N12009, H7N9 and influenza B were 65.1%, 56.5%, 29.2% and 57.1%, while sensitivity of reagent A 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

Clinical University Hospital Virgen de la Arrixaca, Murcia, Spain

Background:
Sepsis is defined as a life-threatening organ dysfunction 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 in clinical practice. Interleukin-6 (IL6) is widely investigated for its first response to the infectious stimulus and Soluble CD14 subtype (Presepin) 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 Presepin 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 Presepin 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 in two groups: A (non-infectious etiology, localized infection or SISS) and B (septic 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 Presepin). The AUCs for the diagnosis of sepsis was 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 Presepin (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:
Presepin 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
assessment of the study of intestinal protozoan in the adult chronic diarrhoea syndrome

E. Lepe Balsalobre1, M. Viloria Peñas1, L. Perpiñ Camacho1, J. Guerrero Montavez2, A. Moro Ortiz2, “Virgen de Valme University Hospital, Seville, Spain, 1Virgen del Rocio University Hospital, Seville, Spain

Background:
Stool analysis is of special relevance in the study of adult chronic diarrhoea 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 independent assessment of the study of intestinal protozoan in the adult chronic diarrhoea 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 suspicion 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 Pennvold 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%.

**Conclusions:** The prevalence study revealed the potential of the Ct value (2.04) for the sensitivity and specificity of the test. 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

F. L. O. Marinho, E. Cueva Mateo, D. A. G. Zauli, Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil

**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 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 patients and individuals. **Methods:** Specimens were collected from patients with suspected 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 intrasample 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 results 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 results 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 results 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 results 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 results of agreement.

**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.**

B. Armondi, L. B. Alvim, E. Cueva Mateo, D. A. G. Zauli, Hermes Pardini Institute (Genetics Division), Vespasiano, Brazil, Hermes Pardini Institute (Research & Development Division), Vespasiano, Brazil

**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 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 co-infection. 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.**


**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 (r² = 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-Bablok 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, -0.4733 to -0.2974) in 187 samples and -0.44 log IU/mL (-1.40 to 0.53) in 20 drug-resistant HBIV.

Conclusion: The VERIS HIV assay is well-suited to monitoring HBV DNA levels in both chronic HBV and drug-resistant HBIV, according to current clinical practice guidelines.

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

T. Chao, H. You. Chang Gung Memorial Hospital Kaohsiung Medical Center, Kaohsiung, Taiwan

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 1° strand cDNA Synthesis system. First PCR of the HCV NS5A was amplified using the pair of primers as follows: sense 5’-AAGAAGGCTCCACCGTGGATC-3’ and antisense 5’-CGCCGAGGGCCATCTGGAC-3’. One microliter from the first PCR reaction were used in the nested PCR with the pair of primers as follows: sense 5’-ATGAGGACTGCTCCACCGC-3’ and antisense 5’-GTAAGAATCCGGGCGC-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 genotype 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; 6.6%), R30Q (n=21; 3.9%), R282K (n=8; 1.48%) and L31H (n=4; 0.74%).

Conclusion: Mutations conferring resistance to HCV NS5A inhibitors are frequent in treatment-naive 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.

**Comparison of different molecular assays to diagnose human respiratory viral infections**

S. Valledor1, E. Machetti-Mareca1, C. Escobar1, J. Gil1, R. Benito1, D. R. Herrero1, 1Facultad de Ciencias, Universidad de Zaragoza, Certest Biotec S.L., Zaragoza, Spain, 2Facultad de Veterinaria, Universidad de Zaragoza, Certest Biotec S.L., Zaragoza, Spain, 3Hospital Clínico Universitario Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, Zaragoza, Spain, 4Hospital Clínico Universitario Lozano Blesa, Facultad de Medicina, Universidad de Zaragoza, IIB Aragón, Zaragoza, Spain, 5Certest Biotec S.L., San Mateo de Gállego, Spain

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 respiratory infections during three years: 2014-2017, comprising different seasonal viruses’ subtypes. All samples were analyzed by VIASURE Respiratory Panel (Certest Biotech S.L.), FTIyo Respiratory Pathogens 21 (Fast Track Diagnostics, FTD) and CLART®PneumoVir (Genomica). The two first assays are lyophilized reagent-based 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:

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**B-077**

**B-079**
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<table>
<thead>
<tr>
<th>VIASURE assay</th>
<th>Fast Track assay</th>
<th>Agreement with reference to CLART®/Pneumovir (Genomica)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. positive samples</td>
<td>No. positive samples</td>
<td>VIASURE vs Genomica</td>
</tr>
<tr>
<td>Influenza A/Influenza H1N1/ Influenza B</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Coronavirus 229E/</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Coronavirus NL63/Coronavirus OC43</td>
<td>24</td>
<td>4</td>
</tr>
<tr>
<td>Paninfluenza 2/Paninfluenza 3/Paninfluenza 4</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Respiratory syncytial virus A/B/Adenovirus</td>
<td>24</td>
<td>22</td>
</tr>
</tbody>
</table>

Total: 108 samples

**Conclusions:** VIASURE Respiratory panel exhibited as good clinical accuracy 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-081**

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

T. Kim1, J. Pitcovski2, H. Yim3, D. Tark4, M. Park5, C. Lee6, S. Hwang6, Y. Kim7, S. Kang8. 1University of Savon, Hwasung, Korea, Republic of; 2MIGAL, Kiryat Shmona City, Israel; 3Korea Zoonosis Research Institute, Cheonbuk National University, Cheonbuk, Korea, Republic of; 4L & K Pharm, Seoul, Korea, Republic of; 5Vaccines Division, National Institute of Food and Drug Safety Evaluation, Chunbuk, Korea, Republic of; 6AVI Officer, Ministry of Agriculture, Food and Rural Affairs, Sejong, Korea, Republic of; 7Animal and Plant Quarantine Agency, Gyefinedub, Korea, Republic of; 8Center for Inflammation, Immunity & Infection, Georgia State University, Atlanta, GA

**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=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 environnemental microbial contamination and to verify disinfection and sterilization procedures**

M. Ferrari. Hospital Lodi, Lodi, Italy

**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
Infectious Disease

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: Di- alysis 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 (HyperD- RYMist® 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 “three 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

w. awoke, armauer hansen research institute, addis ababa, Ethiopia

Abstract

Background: Mycobacterium tuberculosis persist in the human host for decades & reactivation can occur at any point. Becomes dormant and pheno-typically 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 averted development of drug resistance.

Objectives: 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-brook 7H9 with 10% Oleic Acid Albumin Dextrose Catabase as a normal, Phos- phate 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 Fluorescin DiAcetate (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 576 experiments were performed using 4 strains of Mycobac- terium Tuberculosis subcultured on SDW, PBS and 7H9 and. Of these, 324 micro- scopic tests using (106/ZN) acid fastness, 106(FDA) viability, and 106(Sudan black stain) lipid bodies, 108 culture growth reading done. After week fucid fastness, viability and culture growth decreased. 144 phenotypic DST done using MTT as- say. 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 concen- tration observed at the 6th week coincided with the decline in viable bacilli in all media systems, thus attributing this phenomenon to lipid body accumulation alone was difficult.

Keywords: M. tuberculosis, LB%, Drug Tolerance, and MTT Assay.

B-084


G. Ogbonna, E. Hryhorenko, J. Parsells, S. Phonethewpath, L. Sprague.

Ortho Clinical Diagnostics, Rochester, NY


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 on 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 = 0.98x 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 IUL), 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 sum- mary, 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

J. Monteiro, F. Inoue, A. Lobo, M. De Martino, M. C. Ferre, S. Tufik. Asso- ciação Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil

Background: Surveillance cultures are routinely used by public health authori- ties to screen for multidrug resistant bacteria. In the past few decades, the wide- spread use of broad spectrum antibiotics has provided the acquisition of resis- tance 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) organ- isms 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 30th December 2017. All the rectal swab samples were collected from patients admitted from high-risk set- tings or transferred from areas with high rates of MDR organisms. All clinical speci- mens were inoculated onto a selective media (ChromID media, bioMerieux) and incu-
Wednesday, August 1, 9:30 am – 5:00 pm

Infectious Disease

B-087

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

W. Niklinski1, W. Huang1, E. Niklinska2, H. Bui1, B. Bayod1, E. Castro1, S. Kelly2, John H. Stroger Hospital of Cook County, Chicago, IL, 1Vanderbilt University School of Medicine, Nashville, TN, 2Abbott Laboratories, Chicago, IL

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 Pallidum).

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 TPPA. 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

X. Hu1, S. Liao2, B. Ying3, Z. Zhang3, 1West China Hospital, Chengdu, China, 2Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada

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 using microarray followed by qRT-PCR. EHR and follow-up information of patients were collected accordingly. In discovery phase, differentially expressed miRNAs (DEMI) were narrowed down and further selected. In selected and testing phases, models with ‘EHR + miRNA’ and ‘EHR only’ were established using support vector machine. We relieved the overfitting problem with collected. In selection and testing phases, models with ‘EHR + miRNA’ and ‘EHR only’ were established using support vector machine. We relieved the overfitting problem with support vector machine. We relieved the overfitting problem with

Conclusion: Our present study identified 6 exosomal miRNAs as promising non-invasive biomarkers for PTB and tuberculosis meningitis (TB) patients compared with their controls. ‘EHR + miRNA’ model showed a better diagnostic efficacy for TB 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.

Our present study identified 6 exosomal miRNAs as promising non-invasive biomarkers for PTB and tuberculosis meningitis (TB) patients compared with their controls. ‘EHR + miRNA’ model showed a better diagnostic efficacy for TB 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 non-invasive biomarkers for PTB and EBM patients. Combination of exosomal miRNAs and EHR through machine learning algorithm could serve as a feasible approach in promoting TB differential diagnosis, and further prospective validation is required before its clinical utility.

S160 70th AACC Annual Scientific Meeting Abstracts, 2018
Novel rapid quantification method of bacteria in a septic blood sample can produce an effective biomarker for monitoring patient care

H. Niimü, A. Miyakoshi, T. Ueno, M. Wakasugi, Y. Higashi, M. Mori, T. Tabata, H. Minami, A. Takaoka, A. Hayashi, Y. Yamamoto, I. Kita-jima. Toyama University Hospital, Toyama, Japan, Ishikawa Prefectural University, Ishikawa, Japan, Hokkaido Mitsui Chemicals, Inc., Hokkaido University, Hokkaido, Japan

**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.

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

D. A. G. Zauli, E. Cueva Mateo, Hermes Pardihi Institute (Research & Development Division), Vespasiano, Brazil

**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 (r²) 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.
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**

J. Shin¹, H. Lee², S. Kee³, Y. Choi³, Y. Kwon⁴, J. Shin¹, B. Park⁵, S. P. Suh⁶, "Chonnam National University Hospital, Gwangju, Korea, Republic of," Chonbuk National University Hospital, Jeonju, Korea, Republic of, "Mokpo National University, Muan, Korea, Republic of" and

**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**

V. Mackiewicz¹, L. Larrouy¹, F. Damond², K. Peoc’h³, V. Chicha-Cattori⁴, D. Descamps⁵, "Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France, Paris, France, IAME, UMR 1137, INSERM, Université Paris Diderot, Sorbonne Paris Cité, AP-HP, Laboratoire de Virologie, Hôpital Bichat, AP-HP, Paris, France, Paris, France, Biochimie Clinique, Hôpital Beaujon, APHP, HUPVNS Clchly, France, Paris, France

**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 Analyzers 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 HBsAg 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.

**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**

S. Aksaray¹, U. Oral Zeytini², F. Yuce³, S. Daldaban Dincер, O. Yamanlar³, "Haydarpaşa Numune Hospital, Istanbul, Turkey, Association of Public Hospitals Northern Anatolian Region of Istanbul, İstanbul, Turkey

**Background:** Tuberculosis continues to be a major health problem worldwide and also in our country. Rapid and accurate diagnosis is key to controlling the disease. The traditional tests for TB produce results that are either 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.

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

J. Lu, H. Wang. Chang Gung Memorial Hospital,Linkou, Taoyuan, Taiwan

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 shortening length of stay.

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

D. Zhao. Qili Hospital of Shandong University, Jinan, China

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^dim_ 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.
ments. 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.

C. M. M. Oliveira, M. C. da Silva, A. M. Garcia, W. B. de Mello, D. M. V. Gomes, S. V. L. Argolo, G. A. Campana. DASA, Rio de Janeiro, Brazil

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&K903) 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 below

Conclusion: Reactive samples have compromised stability when subjected to successive cycles of refrigeration and exposure to room temperature. The measurement of ODs 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.

B-101
Testing anti-Zika virus NS1 IgA additionally to IgM increases sensitivity in acutely infected patients from regions endemic for flaviviruses

K. Steinhauser1, N. Wilhelm2, O. Sendesch2, W. Schlumberger.1 Institute for Experimental Immunology, EUROIIMMUN AG, Lübeck, Germany, 'EUROIIMMUN US, Inc., Mountain Lakes, NJ

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 Colombina (2015), where ZIKV and DENV are endemic. Patients had been tested positive for ZIKV nucleic acid and anti-DENV IgG during the viremic phase (<5 day). 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 1 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 IgM 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


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 figured on the platform. Of note, we demonstrate simultaneous detection of 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 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 resulted in 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 only 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.
Detection of cytomegalovirus nucleic acid and mycoplasma nucleic acid in alveolar lavage fluid of pediatric patients with respiratory tract infection

X. Cheng, J. Li, Y. Xu, J. Cao. The First Affiliated Hospital of Anhui Medical University, Hefei, China

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 and MP were 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.

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


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 spp for fluorocytosine, fluconazole, voriconazole, amphotericin B, caspofungin and micafungin 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.

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

M. J. Cameron, S. J. Polsinelli, A. A. Emanuele, M. D. Sandison. Lumigen, Southfield, MI

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,EDTA 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,EDTA plasma, 2.0 IU/mL for HBV K,EDTA plasma and 3.8 IU/mL in HBV serum, 4.3 IU/mL for HCV and 30 IU/mL for HIV-1 both in K,EDTA plasma. The standard deviation of the precision for HBV (K,EDTA plasma) was less than or equal to 0.16 for HBV, 0.15 for HCV, 0.16 for CMV and 0.20 Log cp/mL for HIV. The linear range (Log IU/mL) was 2.00-7.01 for CMV, 1.00-9.00 for HCV, 1.56-6.4 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.
Comparison of clinical performance of SD Strep A Ultra Test and SD Strep A Rapid Test for diagnosis of acute bacterial pharyngitis

S. Kim1, W. Choi2. 1Changwon Gyeongsang National University Hospital, Changwon, Korea, Republic of, 2Department of Nursing Science, Kyungsung University, Busan, Korea, Republic of

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 pediatriic 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 spec 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 spec 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.

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

V. S. Moraes1, L. M. SHOLLENBERGER2, W. C. BORGES3, L. C. MEDEIROS4, L. M. V. SIQUEIRA5, R. R. Cruz6, L. A. COUTINHO6, J. V. ASSIS7, M. C. PEDROSA1, C. S. S. PEREIRA1, A. T. RABELLO1, D. A. ALVAREZ1, P. Z. CHAVES1, F. E. FARIAS1, Q. GUIMARAES1, Instituto Rene Rachou, Belo Horizonte, Brazil, 2 Universidad Federal de Ouro Preto, Ouro Preto, Brazil, 3 Instituto Carlos Chagas, Curitiba, Brazil

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 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 phosphatase (ALP). An androsterone derivative with a phosphate was hydrolyzed by ALP, and this deriva-tive was then employed in the enzyme cycling. Consequently, MPB64 could be determined by the accumulated amount of this-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 3×10^4 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.
Performance characteristics of the Alinity i HBsAg Qualitative II, Anti-Hbc II and Anti-Hbs assays utilized for routine laboratory Hepatitis B testing


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 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% (5106/5100) for HBsAg Qualitative II and 99.86% (5162/5160) 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 characteristics for linearity, precision, and bias across the entire measurement range from 2.00 mIU/mL to 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.

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

S. Gannon1, N. Benina1, C. Feldman1, S. Raju1, M. Wang1, K. Falcone1, D. Ziegler1, N. White1, C. Peacock1, J. Lathani1, K. Maddaloni1, J. R. Genzen1, J. J. Redman2, G. Lambert-Messerlian1, D. Grenache1, K. Donaldson1, D. Hawkins1, C. Miller1, R. Radwan1, J. Young1, S. Dolan1, D. Dickson1, C. J. Traynham1, 1Fujirebio Diagnostics Inc, Malvern, PA, 2ARUP Laboratories, Salt Lake City, UT, 3Women & Infants Hospital of Rhode Island, Providence, RI, 4Prescient Medicine, Hershey, PA

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 severe bacterial infections or sepsis, significantly increases synthesis of PCT by C-cells in the thyroid under normal conditions. Systemic inflammatory responses (SIRS), C-reactive protein (CRP), procalcitonin (PCT), tumor necrosis factor (TNF-α), interferon-gamma (IFN-γ) and interleukin-6 (IL-6), are key mediators of the acute inflammatory response. PCT binds to PCT immunoassays, completing the sandwich. The amount of PCT is derived from the luminescence signals generated by adding the substrate AMPPD (3-(4-2’-spirodamanate)-4- methoxy-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 forms the LUMIPULSE G1200 system (n = 8 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 Calcitonin (10 ng/mL), α-CGRP (10,000 ng/mL), β-CGRP (10,000 ng/mL), Salmon Calcitonin (13.2 ng/mL), and Eel Calcitonin (7.5 ng/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, -0.0084, 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.
Clinical Performance of the Bio-Rad BioPlex 2200 Toxoplasma gondii IgM assay

M. Yarbrough¹, E. Theele², H. Hilgart³, A. Gronowski¹. ¹Washington University, St. Louis, MO, ²Mayo Medical Laboratories, Rochester, MN

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 Ig 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 multiple flow immunoassays intended for identification of antibodies to T. gondii, R. bellii 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 consecutively collected specimens, and 2) Archived: 52 archived specimens. The prospective specimens came from patients who had a toxoplasmosis diagnosis as part of routine clinical care; the archived specimens were submitted for anti-T. gondii IgM and IgG 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 ToxoPlasmasma assays (Siemens, Malvern, PA). Performance of the BioPlex 2200 ToRC assay was evaluated by calculating positive percent agreement (PPA) and negative percent agreement (NPA) compared to the Centaur test results.

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 prospectively collected specimens was the population likely false positives. IgM 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 specimens 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: 82.9-98.8%) 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.

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

M. T. Tesfazghi, A. M. Gronowski, M. L. Yarbrough. Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO

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: Two sample populations were utilized: 1) Prospective: 300 consecutively collected specimens and 2) Retrospective: 52 archival specimens from 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.

Results: Among the 300 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% (337/340, 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: 96.9-99%). 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. Conclusion: 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.

Evaluation of FilmArray for Early Diagnosis of Sepsis

M. D. V. Martinho, P. C. M. Koga, I. Pasternak, A. G. Marques, I. Siqueira, A. M. Doi. HOSPITAL ALBERT EINSTEIN, SÃO PAULO, Brazil

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 l’Etoile - France) using the blood culture identification (BCID) panel for the diagnosis 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 gene 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 (false negative) positive culture, but MT showed growth of Roseomonas sp. Partial agreement occurred in 3 cases: one the Biofire identified only Enterobacteriaceae gender and the culture was positive for Citrobacter freundii; two cases there was 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 mecA 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.

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

J. Monteiro, F. Inoue, A. Lobo, M. De Martino, D. R. R. Boscodo, S. Tufik. Associação Fundo de Incentivo a Pesquisa, Sao Paulo, Brazil

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 manufacturer’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=268), C. tropicalis
Transcriptome Differences in Normal Human Bronchial Epithelial Cells in Response to Influenza A pdmH1N1 or H7N9 Virus Infection

C. Huang1, M. Hsiao1, Y. Lin1, H. Wang1, K. Tsao1, L. Lee1, Y. Wu1, R. Kuo1, S. Shih2. Linkou Chang-Gung Memorial Hospital, Taoyuan, Taiwan, *Chang-Gung University, Taoyuan, Taiwan.

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 (MetaCore14, 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 46499 transcripts can be detected on HTA chip, compared with mock control, absolute fold change > 2 (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 remodelaging 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 pathogenetic mechanisms that lead to severe complications.

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

P. L. Rodriguez1, S. McCune1, L. Saka1, J. Engstrom-Melnyk1, J. Osiekci1, E. Marinis1. 1Medical and Scientific Affairs, Roche Diagnostics Corporation, Indianapolis, IN, 2Commercial Education, Roche Diagnostics Corporation, Indianapolis, IN, *Medical and Scientific Affairs, Roche Molecular Systems, Pleasanton, CA.

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 (SerCare Life Sciences, Milford, 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® 8800 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® 8800/8800 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) (9/120). 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.

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

X. Li, S. Zheng, Y. Cheg. First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China.

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 immuneocyte studies indicated that the CD8+ T cells, CD4+CD45RO+ T cells, CD8+CD45RA+ T cells, and T follicular helper (TD) 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 recombiant HBV vaccine’s immune effectiveness.

Acknowledgement

This paper was supported by a grant from the National Natural Science Foundation of China (No. 81672092) and the Major National S&T Projects for Infectious Diseases (2017ZX10202201-002-004).
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Diagnostic performance of Xpert MTB/RIF assay in an intermediate tuberculosis burden setting

S. Kee⁴, H. Lee⁵, J. Shin⁶, Y. Kwon⁷, J. Shin⁸, B. Park⁹, S. P. Suh¹, Chonnam National University Hospital, Gwangju, Korea, Republic of; Chonbuk National University Hospital, Jeonju, Korea, Republic of; Mokpo National University, Muan, Korea, Republic of

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 spuva compared with spot spuva (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.

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

H. Lee¹, S. Kee², J. Shin³, Y. Kwon⁴, J. Shin⁵, B. Park⁶, S. P. Suh⁷, Chonnam National University Hospital, Gwangju, Korea, Republic of; Chonbuk National University Hospital, Jeonju, Korea, Republic of; Mokpo National University, Muan, Korea, Republic of

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 during routine clinical use in an intermediate burden setting.

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.

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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.


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 Immune deficiency 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 log₂ IU/mL. Thirty specimens with known HCV genotypes 1a, 1b, 2b, and 3a were compared over a range of 1.11 to 6.98 log₂ 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 log₂ 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


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 Versaia 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**


**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 EC/EG IQ 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, 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. Seroreversion sensitivity was assessed by testing a commercially available panel. Assay reproducibility was assessed using two reagent lots with 5 member panel. Analytical sensitivity was determined by testing serial dilutions of the WHO 1st International Standard (T. cruzi 1 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**

B. Menge1, O. Klemens1, A. Streit2, O. Sendedsch3, J. Klemens2, K. Steinhagen1,1, EUROIMMUN AG, Lübeck, Germany, 2Max Planck Institute for Developmental Biology, Tuebingen, Germany, 3EUROIMMUN US, Inc., Mountain Lakes, NJ

**Background:** Strongyloides is an infectious disease caused by the nematode Strongyloides. Human infection by Strongyloides stercoralis can manifest with dermatological, intestinal and pulmonary symptoms frequently passing into a chronic disease. Low parasitic loads and discontinuous larval excretion may hamper diagnosis by coproscopy. Serological test systems are more sensitive to detect the infection. Available serological 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) pre-characterized sera. Among these were 55 CE and 52 AE patients, 122 samples of patients with other parasitic infections (including the following species: Fasciola hepatica, Strongyloides stercoralis, Taenia solium, Trichinella spiralis, Schistosoma sp., Plasmudium sp., Toxocara sp., Entamoeba histolytica, Leishmania sp., 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 EAgG and E. multilocularis antigens Em18 and Em95. Bands were automatically evaluated using a commercial software (EUROLimeScan, 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 95% (at 100% specificity). Since the evaluation is challenging, a specific algorithm for species differentiation was developed in the EUROLimeScan software on the basis of the antibody findings. In patients positive for specific anti-Echinococcus sp. 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 EUROLIME-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 sp. and Entamoeba histolytica was observed.

**B-126**

**Recombinant antigens improve sensitivity and allow species differentiation in echinococcosis diagnostics**

L. Schmidt1, T. Scheper1, W. Meyer1, L. Binnenkade1, O. Sendedsch3, J. Warnecke1, 1Institute for Experimental Immunology, affiliated to EUROIMMUN AG, Lübeck, Germany, 2EUROIMMUN US, Inc., Mountain Lakes, NJ

**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:** Methods according to CDC guidelines. For species differentiation, blot techniques using specific recombinant antigens are of increasing significance.

**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 95% (at 100% specificity). Since the evaluation is challenging, a specific algorithm for species differentiation was developed in the EUROLimeScan software on the basis of the antibody findings. In patients positive for specific anti-Echinococcus sp. 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 EUROLIME-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 sp. and Entamoeba histolytica was observed.

**B-127**

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

E. Dovaro1, A. Tomaras2, R. Chamberland1, T. Isbell1, 1Saint Louis University School of Medicine, St. Louis, MO, 2BacterioScan, St. Louis, MO

**Background:** Urinary tract infection (UTI) is common, and urine culture is one of the most commonly performed tests 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 216Ds 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 (216Ds) 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 216Ds to evaluate sensitivity and specificity for UTI detection defined as growth of culture in one or two ureapathogens at concentrations of ≥10,000 CFU/ml. Results: 124 urine samples were evaluated by UA, culture and 216Ds. The 216Ds 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**

J. Song, Y. He, X. Song, Z. Zhao, T. Liu, Y. Zhou, B. Ying. West China Hospital, Chengdu, China

Background: Little knowledge about the biological functions of RP11-37B2.1, a newly-defined long non-coding RNA (IncRNA) molecule, is currently available. Previous studies have shown that r160441, located in the RP11-37B2.1 gene, is significantly associated with tuberculosis (TB) both in a Guanhua and the Gambian populations. Methods: We investigated the influence of several SNPs within IncRNA 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 IncRNA 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 IncRNA RP11-37B2.1 and TB susceptibility was observed in total samples (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: 2.25% vs. CT + TT: 6.92%, respectively) and the calculated 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 IncRNA 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**

S. Ma, K. Cichonski, D. Ryan, A. Bansal, M. Kopnitsky, D. R. Zweigert. ZEUS Scientific, Branchburg, NJ

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 Visel/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®/Marbot®). 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-E352-09E). 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 - Visel/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 - Visel/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 - Visel/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**

R. Krishnamurthy, B. Djiguemde, K. Geary, Y. Le Breton. 3i Diagnostics, Inc., Germantown, MD

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 only on bacterial 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 ultrafiltrin 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 passed 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...
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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.


**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 individuals 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 (30dp) (to verify the therapeutic effect of Epo). All the animals were infected by intraperitoneal route with 10^7 trypanomastigotes Colombian 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 not 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 seen 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-133**

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

A. Seth, R. Chandra. Accurence Medical Labs, Linden, NJ

**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 pathway 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 Accurence 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 results 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?

R. Hawkins, P. De, Tan Tock Seng Hospital, Singapore, Singapore

**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 $\geq$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%.