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Rapid identification of positive blood culture using MALDI-TOF and gel separation technique with short incubation period

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Background: The rapid direct identification positive blood culture using gel separation technique was not satisfactory. This study was to compare identification of positive blood cultures using gel separation with and without short incubation period techniques by MALDI-TOF MS.

Methods and materials: First phase of the study was to determine a short incubation period. Seventy-two bacterial (stored at -80 °C) collected in XiJing Hospital, PR China using a 0.5 McFarland standard suspension were injected into the blood culture bottle. The blood broth with positive signal just flagged were inoculated onto Blood, MacConkey, and Sabouraud agar plates and incubated in automated WASPLab (Copan) system. To establish the fastest visible growth, the image was recorded hourly using automatic photographic mode.

After obtaining the minimal visible growth period, positive blood cultures (100) in our hospital during December 2018 were studied. Two separation methods were used to compare positive identification rate using differential centrifugation and washing (3,500 rpm and 10,000 rpm) gel separation (Insepack) technique to obtain pellet, and same gel separation with short incubation period (determined in first phase of this study). The gold standard identification was determined using pure isolates incubated for 24 h. VITEK MS identification system (bioMerieux) was used. The time for short incubation was determined with identification with > 90% accuracy.

Results: Among 72 strains used to determine short incubation period, average time of Gram-negative (32), Gram-positive (30), and yeasts (10) were 4 h, 6 h and 8 h respectively. The positive identification rate of 100 positive blood cultures using gel separation and gel separation method with short-time incubation period were Gram-negative bacteria (50) 87.6% and 95.4%; Gram-positive bacteria (42) 71.5 and 92.1%; yeast (8) 59.6% and 85.4% respectively. The average TAT time using additional short incubation was shortened by 15.3 h.

Conclusion: Clinical microbiology laboratory with automation system will provide a better diagnostic tool. The new logistics reduced TAT significantly when compared with conventional methods. Direct identification method using gel separation after a short incubation period has greatly improved the accuracy of identification.

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Molecular typing of *Salmonella* spp in pigs with post-weaning infectious enteritis

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Background: Salmonella enterica subsp. enterica is one of the main zoonotic bacteria responsible for cases of salmonellosis in humans and animals, besides, it is one of the most widely spread pathogenic organisms worldwide. Pigs can be infected with multiple Salmonella serotypes and their presence can be associated with the geographical distribution of pig production and bacterial resistance through the use of antibiotics as animal growth promoters. Pork meat plays an important role in the transmission of salmonellosis to food and has been associated with its presence in humans. Objective: The main objective of this research was to isolate, identify and molecularly typify Salmonella species associated with infectious enteritis in post-weaning pigs and antimicrobial resistance (AMR) of a commercial swine farm.

Methods and materials: Methods: Samples were collected with swabs from diarrheic post-weaning pigs (rectal). Selective selenite broths were used, and selected samples were cultured in Hektoen and Mac Conkey agar plates. *Salmonella* like-colonies were stabbed and biochemical tests for confirmation were conducted

Results: PCR was carried out in 16 S rRNA genes for *Salmonella* like-colonies compatible with genus *Salmonella* spp and PCR amplicon profiling was generated on positive samples. The phylogenetic analysis was conducted using **MEGA** version 7. Four colonies out of 15 (4/15, 26.6%) were identified as positive for *Salmonella enterica*. This investigation belongs to the first set of stages planned to design eubiotic mixtures for the treatment of this disease.

Conclusion: Results obtained in this investigation confirmed the presence of *Salmonella enterica* subsp. enterica serovar Heidelberg, *Salmonella enterica* subsp. enterica serovar *Paratyphi*, and *Salmonella enterica* subsp. enterica serovar *Typhimurium* typified by molecular diagnosis techniques in post-weaning pigs.

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Dynamics of the level of interleukins in the blood of children undergoing hemorrhagic fever with renal syndrome

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Background: Hemorrhagic fever with renal syndrome (HFRS) is caused by hantavirus, which does not have a direct cytopathic effect on endotheliocytes, but serves only as an initiator of immune inflammation. Cytokines play a leading role in the pathogenesis of

many infectious diseases, including HFRS. In the literature available to us, we have not seen a single publication devoted to the dynamics of cytokines in children with HFRS.

Methods and materials: The study included 147 children with HFRS hospitalized in an infectious diseases hospital in Ufa. Patients were conditionally divided into 2 groups. The first group consisted of 73 patients who were treated with a diagnosis of HFRS of moderate severity. The second group included 74 patients with a diagnosis of severe HFRS. The age in the two groups was 5–17 years. The average age is 11.1 ± 1.6 y.

Results: In children with HFRS, there is an increase in the concentration of pro-inflammatory cytokines IL-1β and TNFa, starting from the febrile period. The peak increase in these cytokines is observed in the oliguric period. During the period of polyuria, the level of pro-inflammatory cytokines decreases. Moreover, during the recovery period, their difference from the control group is not statistically significant. The level of inf and inf-γ in children with HFRS decreases since the febrile period, the greatest decrease is observed in the oliguric period with severe HFRS. In the polyuric period, the level of inf and inf-γ rises, but with a severe form of the disease, their level is statistically significantly higher than in the control group even during the recovery period. The concentration of the anti-inflammatory cytokine IL-10 in the febrile period tends to increase, a statistically significant increase is noted in the oliguric period and continues to increase in the polyhuric period. In the convalescence phase, the level of IL-10 is not statistically different from the control group, while in adult patients, the level of IL-10 in the convalescence period is significantly higher than in the control group.

Conclusion: Thus, the content of cytokines in children with HFRS depends on the period and severity of the disease.

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Reproducibility of Roche cobas HIV-1, HBV and HCV real-time PCR assays on cobas 4800 in comparison with COBAS AmpliPrep/COBAS TaqMan assay equivalents

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Background: Quantitative real-time PCR for viral load testing is vital for the diagnosis, disease monitoring and management of important blood-borne viral infections. Laboratories needing to switch to a newer real-time PCR system in favour of technological advancements require an evaluation on the reproducibility of its tests compared to the established, currently in-use system before their implementation as replacement viral load tests. In this study, we determine the reproducibility of the Roche cobas HIV-1, HBV and HCV real-time PCR tests on the newer cobas 4800 system when compared to the COBAS HIV-1, HBV and HCV (version 2.0) tests on the COBAS AmpliPrep/COBAS TaqMan system.

Methods and materials: Twenty-one undiluted EDTA-plasma samples were selected for each assay pair by convenience sampling from known positive clinical specimens for this evaluation study. The procedures for nucleic acid extraction and real-time quantitative PCR were performed according to the manufacturer's instructions. Reproducibility was determined by measuring correlation using regression analysis and agreement using Bland-Altman analysis of only valid titer pairs that were within the linear ranges of each assay. IBM SPSS Statistics software version 21 was used for both analyses.

Results: The number of samples that were out of the linear range and were excluded from the analysis was 4, 5 and 1 sample for HIV-1, HBV and HCV tests, respectively. Cobas 4800 HIV-1, HBV and HCV tests demonstrated a good correlation with COBAS AmpliPrep/COBAS TaqMan HIV-1, HBV and HCV (version 2.0) tests with regression values of 0.9924, 0.9795 and 0.9284, respectively. All sample data recorded for all three assay pairs were found to be within the significant range of ± 1.96 SD.

Conclusion: The performance of the cobas 4800 HIV-1, HBV and HCV tests were reproducible when tested with the COBAS AmpliPrep/COBAS TaqMan HIV-1, HBV and HCV version 2.0 tests, within each of the respective test's linear ranges. The cobas HIV-1, HBV and HCV real-time quantitative PCR tests on the cobas 4800 system are suitable as replacement viral load tests in our setting with acceptable performance compared to the currently in-use system.

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An extraction-free amplification strategy for rapid and ultrasensitive detection of malaria

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Background: As a serious life-threatening disease, malaria cause more than 400,000 deaths every year. The control and elimination of malaria is still a global challenge. Accurate diagnosis and prompt treatment of malaria infections is the key to the control of malaria. Traditional microscopy and rapid diagnostic test offer poor sensitivity, which can hardly identify asymptomatic infections. Quantitative PCR can provide highly sensitive results, but rely on time-consuming procedures and expensive instruments. A sensitive, rapid, simple and affordable detection method is urgently required for the active screening of malaria.

Methods and materials: We develop a capture and ligation-assisted loop-mediated isothermal amplification (CLIP-LAMP) strategy for the rapid and ultrasensitive detection of *Plasmodium*. 18S rRNA of *Plasmodium* is released after cell lysis and hybridized with the ligation probe, as well as the capture probe modified on the surface of magnetic nanoparticles. Subsequently, product of the sandwich hybridization is separated and enriched under the external magnetic field. Double stranded template is formed after ligation for the isothermal amplification. We perform the test of parasite diluted with whole blood lysate to evaluate the sensitivity of the method. To further investigate the performance of the method in active screening of malaria, we detect dried blood spots of volunteers and compare the results with standard detection methods (microscopy, rapid diagnostic test, and quantitative PCR).

Results: CLIP-LAMP shows a wide linear range from 100 to 0.001 p μL^{-1} with a detection limit of 0.0004 p μL^{-1}. The whole detection process can be completed within 2 h. In the test of dried blood spots, all the results are consistent with standard quantitative PCR. Microscopy identified 3 of the 11 positive samples, while rapid diagnostic test identified only 1 positive sample.

Conclusion: As an extraction-free isothermal amplification strategy for RNA, CLIP-LAMP eliminates the reliance of complicated