2086. Evaluation of a Multiplex PCR Assay with Molecular Beacon Probes to Rapidly Detect Bacterial Pathogens Directly in Bronchial Alveolar Lavage (BAL) Samples from Patients with Hospital-Acquired Pneumonia (HAP)

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Background. Blood cultures (BC) fail to detect a pathogen in most patients with neutropenic fever (NF). We examined the performance of the Karius next generation sequencing platform on plasma samples compared with blood cultures and routine bacterial culture(s) among patients with NF.

Methods. Blood samples were collected at baseline and during the course of hospitalization from patients with NF. Diagnostic culture was performed on all blood samples. A subset of these samples were submitted to the Karius Clinical Laboratory to perform a targeted next-generation sequencing panel.

Results. At the end of the first week, 47 patients (76%) were positive for at least one pathogen on culture. Of the remaining 15 patients, 2 were negative for all culture and 13 remained un-detected. Karius test was positive in 12 patients. Of them, 4 had potential detections of M. tuberculosis, N. gonorrhoeae, S. aureus, and C. difficile. The Karius test was negative in 34 patients. Of them, 21 were negative for all culture and 13 remained un-detected. Karius test was positive in 12 patients. Of them, 4 had potential detections of M. tuberculosis, N. gonorrhoeae, S. aureus, and C. difficile. The Karius test was negative in 34 patients.

Conclusion. A multiplex PCR assay with molecular beacon probes can be used to rapidly detect bacterial pathogens in BAL samples from patients with NF. This assay can be used in routine clinical practice to detect bacterial pathogens in patients with NF. The assay has sensitivity and specificity similar to that of culture. The assay is cost-effective and can be used as a rapid test to detect bacterial pathogens in patients with NF.

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2085. Metagenomic Analysis reveals Importance of Anaerobes in Development of Infectious Diseases

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Background. Necrotizing soft-tissue infections (NSTIs) are a rapidly spreading necrotizing soft-tissue infection (NSTI). Based on culture data, the microbiology of both infections are similar ranging from a self-resolving abscess to a rapidly spreading necrotizing soft-tissue infection (NSTI).

Aim. The objective of this study was to better understand the impact of anaerobes in NSTIs.

Method. Samples were collected from patients with NSTIs. Their presence may be one of the determining factors that favor the NSTI pathology over the easily-treated abscess. If true, this should lead to more aggressive treatment of NSTIs. Their presence may be one of the determining factors that favor the NSTI pathology over the easily-treated abscess. If true, this should lead to more aggressive treatment of NSTIs. Our objective was to determine the importance of anaerobes in NSTIs.

Results. Blood cultures (BC) fail to detect a pathogen in most patients with neutropenic fever (NF). We examined the performance of the Karius next generation sequencing platform on plasma samples compared with blood cultures and routine bacterial culture(s) among patients with NF.

Methods. Blood samples were collected at baseline and during the course of hospitalization from patients with NF. Diagnostic culture was performed on all blood samples. A subset of these samples were submitted to the Karius Clinical Laboratory to perform a targeted next-generation sequencing panel.

Results. At the end of the first week, 47 patients (76%) were positive for at least one pathogen on culture. Of the remaining 15 patients, 2 were negative for all culture and 13 remained un-detected. Karius test was positive in 12 patients. Of them, 4 had potential detections of M. tuberculosis, N. gonorrhoeae, S. aureus, and C. difficile. The Karius test was negative in 34 patients. Of them, 21 were negative for all culture and 13 remained un-detected. Karius test was positive in 12 patients. Of them, 4 had potential detections of M. tuberculosis, N. gonorrhoeae, S. aureus, and C. difficile. The Karius test was negative in 34 patients.

Conclusion. A multiplex PCR assay with molecular beacon probes can be used to rapidly detect bacterial pathogens in BAL samples from patients with NF. This assay can be used in routine clinical practice to detect bacterial pathogens in patients with NF. The assay has sensitivity and specificity similar to that of culture. The assay is cost-effective and can be used as a rapid test to detect bacterial pathogens in patients with NF.

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No cross reactivity was observed with any of the malarial species tested. Babesia M01, Babesia duncani and all bacterial isolates were negative by the BMPCR. Intra-run, inter-run and day to day reproducibility of the assay was 100%.

Conclusion. The B. microti real time PCR assay developed by Northwell Health Laboratories is rapid, sensitive, specific and reproducible. With the sample to result turn around time of 2.5 hours and hands on time of only 5 minutes per sample, BMPCR can be used as screening assay for B. microti in clinical laboratories.

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2089. A Novel Diagnostic Method for Malaria Using Loop Mediated Isothermal Amplification (LAMP) and MinION Nanopore Sequence

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Background. Simply and accurately diagnostic tool for Malaria is required for clinical diagnosis and epidemiological survey. We have developed a novel diagnostic tool for Malaria using loop mediated isothermal amplification (LAMP) with MinION nanopore sequence.

Methods. In this study, we have designed human Plasmodium parasites-specific LAMP primers targeting for the lesion of 18S rDNA gene, which were locating on the conserved sequences across all five Plasmodium species: Plasmodium falciparum, P. vivax, P. ovale (P. wallikeri and P. crusitis), P. knowlesi and P. malariae, containing each species-specific sequence within F1-F1 primer pairs. The sensitivities were evaluated using 10-fold serial diluted plasmodium harboring the sequences of 18S rDNA. We also applied our protocol to human blood samples collected and stored with FITA elite cards derived from 30 Malaria patients, who are clinically diagnosed as Malaria in Indonesia. Its analytical sensitivities and specificities were also evaluated while comparing the results of previously described nested PCR methods. Finally, we performed amplicon sequencing of our LAMP methods using MinION nanopore sequence to identify each Plasmodium species.

Results. Our LAMP method could amplify all targeting 18S rDNA gene on conserved DNA fragments harboring the sequences of 18S rDNA. In this study, we have amplified Plasmodium species based on the sequence analysis with MinION were also consistent with the sequence of each constructed plasmid and could consistently confirmed its analytical sensitivities and specificities were also evaluated while comparing the results of previously described nested PCR methods. Finally, we performed amplicon sequencing of our LAMP methods using MinION nanopore sequence to identify each Plasmodium species.

Conclusion. Our innovative diagnostic technology with LAMP and MinION could become a powerful tool for identification of Plasmodium parasites even in resource-limited situation.

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2089. Accelerating Time to Pathogen-adapted Antibiotic Treatment through Culture-independent Antimicrobial Susceptibility Testing in Patients Suffering from Sepsis

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Background. Accurate and fast pathogen identification and consecutive antimicrobial susceptibility testing (AST) is of vital importance for patient outcome in patients suffering from sepsis.

Methods. The Accelerate Pheno® system is a new, fully automated, culture-independent diagnostic method for both pathogen identification (ID) and antimicrobial susceptibility testing (AST). We analyzed positive blood cultures from critically ill patients with new onset of sepsis according to the new sepsis guidelines, using both conventional standard methods (VITEK, MALDI-TOF) and Accelerate Pheno® systems. ID/AST results of the Accelerate Pheno™ system were not reported to treating physicians as part of our internal evaluation process.

Results. Accelerate Pheno® system correctly detected 74 pathogens [Gram-negative (GN) (n = 27), Gram-positive (GP) (n = 47)] straight out of 84 positive blood culture bottles. Gram-negative (GN) pathogens were identified as E. coli (n = 15; concordance rate 100%), E. cloacae (n = 3, 100%), E. cloacae (n = 2; 50%), P. mirabilis (n = 1; 100%) and P. aeruginosa (n = 1; 33%). Gram-positive pathogens were identified as CNS (n = 24; 82.6%), S. aureus (n = 15; 88.2%), E. faecium (n = 6; 100%) and E. faecalis (n = 2; 100%). The Accelerate Pheno® system generated a GN-AST result in 70.4% (19 of 27 samples) and a GP-AST result in 61.7% (29 of 47 samples) when compared with routine AST. Growth control, analysis and mechanical failure led to reduced results in comparison to conventional ID/AST. Accelerate Pheno® delivered correct MIC results for most of the panel antibiotics [e.g., meropenem: 83.3%; gentamicin: 88.9%, etrapenem: 100%].

Conclusion. The use of the Accelerate Pheno® system significantly improved time to-ID/AST and would have led to reduced time-to-treatment in patients suffering from sepsis if results would have been reported. The system currently lies in some weakness in the detection of polymicrobial and streptococcal infections but due to the short hands-on-time, culture-independence and fast generation of results, it represents a promising new diagnostic method for the consecutive antibiotic treatment of septic patients.

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2090. T-Cell Immunity Panel Measures CMV Specific CD4 and CD8 T-Cell Responses

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Background. Infection and disease from human cytomegalovirus (CMV) is a major complicating factor for both solid organ and hematopoietic stem cell transplant recipients. Antiviral therapy is often used to control CMV infections, but presents problems of toxicity, antiviral resistance and excessive costs. Currently, treating physicians are limited in the information and data available to assess a patient’s ability to control a potential CMV infection post-transplant. Recent studies have shown that measuring a patient’s CMV specific T cell mediated immunity may provide valuable information in determining CMV infection/disease in transplant patients and may aid in determining which patients need antiviral therapy.

Methods. For this purpose, a flow cytometry assay was developed to determine the percentages of CD4+ and CD8+ T cells that respond to stimulation with CMV antigens based on cellular activation surface marker CD69 in conjunction with IFNγ, TNFα and IL-2 cytokine production. Three CMV antigens were used to assess patient immunity; a whole viral lysate, a peptide pool of p65, and a peptide pool of IE-1.

Results. Our data indicate that CD8 T cells respond primarily to the p65 and/or IE-1 peptide pools while the CD4 T cells respond primarily to the viral lysate. Detection of both CD4 and CD8 responding populations at levels above background, ≥ 0.2% of the parent population, indicates that a patient’s immune system has previously been exposed to CMV. Viable detection limits were 1.0 × 10^3 CMV seropositive samples demonstrated immune responses for all 23 samples above 0.2% for at least one of the three intra-cellular cytokines and at least one of the three CMV antigens. Validation of five CMV seronegative samples demonstrated immune responses below 0.2% (when excluding underling, unrelated immune responses). Included for each sample is a positive (Staphylococcal Enterotoxin type B) control to assess patient’s overall ability to mount an immune response and negative (media) control to capture the presence of an underlying immune response.

Conclusion. This assay evaluates a patient’s pre-existing CMV specific T cell immunity and their global T cell function.

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2091. Application of Laser Light Scattering Technology in Rapid Diagnosis of Urinary Tract Infections and Antimicrobial Susceptibility Testing in a Tertiary Children's Hospital

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Background. Timely and accurate microbiology testing is crucial in the diagnosis and management of urinary tract infections (UTIs). The ability to rapidly screen for potential UTIs can lead to early rule out and judicious use of antimicrobial therapy. This study examines the application of laser scattering for bacterial detection and antimicrobial susceptibility testing (AST) directly from urine.

Methods. Residual urine samples collected for routine culture were tested using the BacterioScan™ 216Dx™ UTI System and 216R AST System. Continuous collection of Urinary tract infection patients generated growth curves and bacteria characterization. Whether the sample was likely positive or negative for bacteria. Further curve analysis ruled out mixed flora at lower concentrations, and “qualified” samples were identified directly on MALDI-TOF MS. AST for ampicillin, cefazolin, ceftriaxone and ciprofloxacin was performed concurrently on the instrument. Samples were incubated for up to 16 hours with results available as early as 2 hours.