Development and Characterization of a Synthetic DNA, NUversa, to Be Used as a Standard in All Quantitative PCR Reactions for Molecular Pneumococcal Serotyping

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Results. 210 urine samples were tested. After 3 hours of incubation on the BactiScan, 70 (33.3%) and 140 (67.7%) urine samples were reported as positive and negative for bacterial growth, respectively. 136/140 (97.1%) of the negative samples were either no growth (67.6%) or insignificant (32.4%) growth by culture. The remaining 4 (2.9%) were catheter (3) or surgical (1) samples that grew <10K CFU/mL. Susceptibility testing confirmed that the assay SD, 33/37 (47.1%) samples were tested on the 216R AST System; 37/70 (52.9%) samples omitted by curve analysis showed no or questionable significant growth by culture. Comparator data were available for 26/33 samples. Ampicillin and ceftiraxone demonstrated categorical agreement of 100%, while cefazolin and ciprofloxacin had 96% and 88% agreement, respectively, with 4% major errors for ceftazolin and 12% minor errors for ciprofloxacin.

Conclusion. The 216R UTI System could be utilized as a screening platform to rule out UTIs within 3 hours, with AST available after an additional 2–6 hours for suspect UTI positive samples. This could potentially prevent unnecessary antibiotic therapy.

Disclosure. All authors: No reported disclosures.

2092. Development and Characterization of a Synthetic DNA, NUversa, to Be Used as a Standard in All Quantitative PCR Reactions for Molecular Pneumococcal Serotyping

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Session: 237. Diagnostics - Novel Diagnostics
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Background. Identification of Strepococcus pneumoniae (Spn) and its more than 90 serotypes is routinely conducted by culture and Quellung reactions. Quantitative (qPCR) based technologies developed for molecular detection, including lrtA assay, and assays targeting 78 serotypes. Reactions require genomic DNA from every target to prepare standards, which can be time consuming. In this study we have developed a synthetic DNA molecule as a surrogate for genomic DNA and present new single-plex qPCR reactions to increase molecular detection to 94 pneumococcal serotypes.

Methods. Single-plex qPCR reactions (N=11) that detect 16 pneumococcal serotypes/serogroups were developed and concentration of primer and probe optimized to obtain 100% efficiency between 96 and 110%. Spectroscopy for the target serotype/serogroup of these new reactions was investigated using a collection of strains belonging to our laboratory and strains kindly donated by the “StrepLab” at CDC. A synthetic DNA molecule (NUversa, ~8.2 kb) was then engineered to contain all available qPCR targets for serotyping and lrtA. NUversa was cloned into pUC57 Amp-modified to generate pNUversa (~10.2 kb). Standards prepared from pNUversa and NUversa were compared against standards made out of genomic DNA.

Results. Specificity of these new reactions was confirmed, and after optimization, the obtained limit of detection (LOD) was between 2 and 20 genome equivalents/reaction. Molecular studies demonstrated that linearity [NUversa (R²>0.982); pNUversa (R²>0.991)] and efficiency of qPCR reactions using synthetic DNA were similar to those utilizing chromosomal DNA (R²>0.981). Quantification, however, with plasmid pNUversa (Y=Int-43.0 1.12) was affected whereas using synthetic NUversa (Y=Int-48.3 1.07) was comparable to genomic DNA (Y=Int-39.9 ± 0.62).

Conclusion. We validated new single-plex reactions that, together with published qPCR reactions, now make possible to detect and quantify 94 pneumococcal serotypes/serogroups. NUversa can be utilized as a control in most, if not all, published single-plex qPCR reactions, for the identification (i.e., detection), and quantification (i.e., genome equivalents) of pneumococcal serotypes.

Disclosure. All authors: No reported disclosures.

2093. The DISCOVER Trial: Application of the Karius Plasma Next-Generation Sequencing Test for Pathogen Detection in Stem-Cell Transplant Patients

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Background. The diagnosis of infections in stem-cell transplant (SCT) patients is challenging. There is a need for improved diagnostics that can quickly and accurately detect a broad range of viruses, bacteria, and fungi that can infect these patients to guide their therapy.

Methods. We enrolled 20 patients in a prospective study evaluating the Karius plasma next-generation sequencing (plasma NGS) test to detect infections in SCT patients. Patients had baseline plasma samples drawn prior to transplant followed by weekly collections during engraftment and at onset of febrile episodes. Samples were transferred to the Karius CLIA/CAP laboratory (Redwood City, CA) where cell-free DNA was extracted from plasma and NGS performed. Human sequences were removed and remaining reads were aligned against a curated pathogen database. Organisms present at a significance level above a predefined threshold were reported.

Results. Cytomegalovirus (CMV) was identified in 12/20 patients. Using a nearest-neighbor method, 61 pairs of observations were identified for a comparison of plasma NGS and CMV qPCR. For plasma NGS, 12% of samples had positive agreement (PPA) between plasma NGS and CMV qPCR with 84.1% (37/44). PPA was 100% (20/20) when values below the lower limit of quantitation (<137 IU/mL) for CMV qPCR were excluded. In addition to CMV, plasma NGS detected EBV, HHV6B, BK virus, and adenovirus. Plasma NGS also detected pathogens prior to conventional tests being ordered in two patients with subsequently confirmed acute infections; Staphylococcus aureus was detected one day prior to blood culture in one patient and Chlamydia trachomatis 30 days prior to targeted testing in a second patient. These two cases highlight the potential of plasma NGS to detect pathogens in SCT patients early when used as a monitoring tool.

Conclusion. Karius has developed a novel NGS plasma test that can simultaneously identify pathogens in SCT patients. The test had 100% concordance with CMV qPCR above the lower limit of quantitation. Further work is ongoing to determine the lower limits of detection for the plasma NGS test. Using NGS to monitor SCT patients for infection could permit earlier detection of pathogens, enabling earlier targeted therapy for this vulnerable population.


2094. Futility of Centor Score (CS) for Predicting Group A Streptococcal (GAS) Pharyngitis in an Adult Hyper-endoemic Native American (NA) Population

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Background. Prevalence of GAS pharyngitis among adults is 5% to 15% in the general population. Methods using clinical criteria and laboratory testing to diagnose GAS have not been evaluated in Native American (NA) populations with a higher prevalence than the general population.

Methods. Prompted by an apparent increase (10-15x above national rates) in inner-city native NA adults in 2016 we began a comprehensive epidemiologic study of GAS. Part of this evaluation included GAS CS. From January to March 2017, we collected a Centor score (CS), throat swab for culture and rapid antigen test (RAT) for all adults ≥18 years presenting with sore throat. For comparison, we also reviewed our electronic health record (EHR), identifying all adults with RAT on file from Jul. to December 2016.

Results. From July to December 2016, 224 (33.5%) adults with sore throat had a positive RAT. From January to March 2017, 268 adults had RAT and culture performed: 86 (32.1%) and 83 (31.7%) were positive by RAT and culture, respectively. Comparing adults 18–44 years and ≥ 45 years, odds of culture positive GAS pharyngitis for young age group were 2.00 (2.56–3.88, P = 0.023). RAT alone was 75.4% sensitive and 88.0% specific. Comparing adults18–44 years to ≥ 45 years, RAT alone was less sensitive (70.1% vs 94.4%) and less specific (86.6% vs 90.6%) in the younger group. Adding RAT plus CS (alone) to the model increased R of CS alone, the addition of CS did not significantly change specificity (91.3% vs. 88.0%) or sensitivity (74.7% vs. 75.3%). A higher CS increased the odds of a positive GAS culture. Tonsillar exudates (89.9%) and fever (51.9%) were the most and least sensitive criteria, respectively. Absence of cough (50%) and absence of malaise (34.9%) were the most and least specific criteria, respectively.

Conclusion. GAS was confirmed in > 30% of cases by RAT on both retrospective review of the EHR and prospectively via RAT or culture. These rates are significantly higher than what is reported in general population. Young age was associated with culture positive GAS. The high sensitivity of exudates and high specificity of absence of cough indicates these criteria may be helpful in deciding which adults are most likely to have GAS. Higher CS did increase odds of GAS positive culture, but the addition of CS to RAT did not significantly alter sensitivity or specificity in this population.

Disclosure. All authors: No reported disclosures.


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Background. Non-interferon based treatment regimens have transformed the therapeutic paradigm for hepatitis C infection. Universal one-time hepatitis C anti-body testing is recommended by the CDC (2013) guidelines.