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Background. Bone and Joint Infections (BJI) have high morbidity and are difficult to treat infections. Culture-based diagnosis is limited in its ability to recover fastidious bacteria and because several organisms can be involved; culture times of up to two weeks may be necessary for certain bacteria. The sensitivity of culture is also negatively impacted by antibiotics received before surgery. Alternatively, molecular methods offer a promising improvement for the diagnosis of BJI. The goal of this study was to evaluate a development version of Biofire® Bone and Joint Infection (BJI) Panel (bioMerieux SA, BioFire Diagnostics, LLC) using synovial fluid samples.

Methods. 121 synovial fluid specimens were collected from patients with suspected bone and joint infection in a pilot evaluation. All specimens were collected and tested in culture by the sites using their standard of care practices; in parallel, a leftover volume of 200 µL was tested on the BJI panel. BJI panel results were then compared with culture and discordant results were investigated using a comparator assay (PCR/sequencing).

Results. 49 synovial fluid specimens (40%) were positive by culture vs. 72 with the BJI panel (59%). Of the 97 positive detections by the BJI panel, 58 were concordant with culture; the 39 additional organism detections were in majority confirmed by PCR/sequencing. Lastly, two false negative results corresponding to the same sample are under investigation.

Conclusion. The BJI Panel was able to identify most of the pathogens detected by culture. The majority of additional detections observed were confirmed by PCR/sequencing. While sites are currently enrolling more synovial fluids samples, these preliminary data suggest that a multiplexed molecular test may be more sensitive than culture to detect pathogens in synovial fluid specimens.

The data presented in this abstract have not been reviewed by FDA or other regulatory agencies for In Vitro Diagnostic use.

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2291. A Real-Time Sequencing Approach for Simultaneous Metagenomic and Transcriptomic-Based Diagnosis of Infectious Diseases

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Background. Recent studies have demonstrated the utility of metagenomic next-generation sequencing (mNGS) and RNA gene expression sequencing (RNA-Seq) for identifying causes of infections. Although these approaches have been largely tested to date using established sequencing platforms such as the Illumina HiSeq, the use of nanopore sequencing on the MinION sequencer (Oxford Nanopore Technologies) is attractive given rapid library preparation and real-time analysis of sequencing data resulting in accelerated sample-to-answer turnaround times.

Methods. We have developed a rapid molecular concatemerization library approach to increase the throughput of the nanopore sequencer analysis for metagenomic and RNA-Seq approaches. We have also developed a pipeline (SURPI*rt*, "Sequence-based ultra-rapid pathogen identification, real-time") that allows for real-time, simultaneous metagenomic and transcriptomic analyses on the same sample.

Results. With the use of molecular concatemerization library approach, we show that metagenomic and transcriptomic data generated on the MinION are comparable to those on the Illumina platform, yet can be collected and analyzed in significantly less time (6 hours vs. 2–3 days).

Conclusion. Here we demonstrate simultaneous metagenomic and RNA-Seq analyses on a nanopore-based sequencing platform with real-time analysis of results. We foresee that this approach could be leveraged into a rapid screening test for diagnosis of infectious diseases in both hospital and field settings.

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2292. Comparison of Molecular Assays for the Diagnosis of Pertussis

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Background. Pertussis is a vaccine preventable disease caused by *Bordetella pertussis* with highest mortality observed in infants. Rapid diagnosis allows prompt treatment and administration of prophylaxis to those at high risk of severe disease. Molecular assays are commonly used for diagnosis because of the long turn-around time and reduced sensitivity associated with culture of samples obtained >2 weeks after symptom onset. We compared the workflow and performance of two molecular assays for the detection of *B. pertussis* from nasopharyngeal (NP) swab specimens.

Methods. NP swabs in universal transport media submitted to Cleveland Clinic for *B. pertussis* testing are routinely tested by the AmpliVue Bordetella assay (Quidel). The AmpliVue utilizes helicase-dependent amplification targeting the insertion sequence IS481 and detection in a lateral flow device. Remnant specimens (n = 112) were stored at -70° C until IRB approval was obtained for this study. The Simplexa™ Bordetella Direct PCR assay (DiaSorin Molecular) targeting IS481 for detection of *B. pertussis* and IS1001 for identification of *Bordetella parapertussis* was performed on the LIAISON MDx instrument. The Simplexa and AmpliVue results were compared. To arbitrate discordant *B. pertussis* results or positive results for *B. parapertussis* (not included in the AmpliVue assay), samples were sent to DiaSorin for sequencing. Sensitivity and specificity were determined for each assay's detection of *B. pertussis* based on sequencing as the reference method for discordant samples.

Results. Positive results for *B. pertussis* were detected for 14 specimens by AmpliVue and 18 specimens by Simplexa. Discrepancy analysis by sequencing confirmed 4 *B. pertussis* positive specimens detected only by Simplexa and one false-positive result for each assay. The sensitivities of AmpliVue and Simplexa were 76.5% and 100%, respectively. The specificity of both assays was 98.9%. Positivity rates were 27% for 48 children ≥1 year, 4% for 25 infants, and 8% for 39 adults tested. The Simplexa *B. parapertussis* target detected in one child's specimen was confirmed by sequencing.

Conclusion. Compared with AmpliVue, the Simplexa assay required less hands on time and provided detection of more specimens containing *B. pertussis*.

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2293. Evaluation of Three Rapid Molecular Assays for the Detection of Group A *Streptococcus*

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Background. Group A *Streptococcus* (GAS), the primary causative agent of bacterial pharyngitis, is most commonly diagnosed with a rapid antigen test performed at the point of care followed by bacterial culture, if negative. Final test results may not be available for 24–72 hours, which can delay the time to therapy and cause patients to miss additional work or school days. Recently, rapid molecular tests, including some that are CLIA-waived, have become available allowing providers to obtain results within a timeframe similar to rapid antigen tests, but with accuracies comparable to traditional culture. The purpose of this study was to evaluate the performance of the Alere™i Strep A test, Roche cobas® Strep A test, and the Cepheid Xpert Xpress Strep A Test (RUO Version) compared with the OSOM Group A Streptococcus rapid antigen test and traditional bacterial culture. All molecular tests are either currently or in the process of obtaining CLIA-Waived status and can be completed in less than 25 minutes.

Methods. The current testing process in our healthcare system (AdvocateAuroraHealth) is to collect oropharyngeal swabs with both a traditional swab and an ESwab (Copan). The traditional swab is used for rapid antigen testing