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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

and the ESwab is submitted to the microbiology laboratory for bacterial culture, if indicated. Residual ESwab specimens were de-identified, cultured, and tested using the Alere and Roche molecular assays (at the time of de-identification the result of the GAS rapid antigen test that was performed on the same patient at the time of ESwab collection was noted). Following testing, ESwab specimens were frozen and tested on the Cepheid molecular assay within 6 months. In total 194 specimens were compared.

Results. Specimens positive by culture or in two of three molecular assays were considered true positives. The results can be seen in the Table below.

Test Type	Specimen Statistics				True Pos	False Pos	True Neg	False Neg	Total Correct	Positive % Agreement	Negative % Agreement	Total Agreement
	# Tested	# Positive	# Negative	Specimen								
Rapid Antigen	194	60	134	Traditional Swab	52	2	132	8	184	86.67%	98.51%	94.85%
Traditional Culture				ESwab Medium	56	0	134	4	190	93.33%	100%	97.94%
Alere Molecular				ESwab (used swab not medium)	59	0	134	1	193	98.33%	100%	99.48%
Roche Molecular				ESwab (200 µL ESwab medium)	60	0	134	0	194	100%	100%	100%
Cepheid Molecular				ESwab (300 µL thawed ESwab medium)	60	0	134	0	194	100%	100%	100%

Conclusion. All molecular tests were more sensitive than antigen testing and culture and could be completed in a timeframe similar to the rapid antigen test. Replacing traditional GAS diagnosis with rapid GAS molecular assays will allow providers to make definitive clinical decisions in near real-time.

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2294. Evaluation of the Karius Plasma Next-Generation Sequencing Cell-free Pathogen DNA Test to Determine the Etiology of Infection and Impact on Anti-Microbial Management in Patients with Severe Neutropenia and Fever

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Background. Standard microbiological testing (MT) fails to identify a pathogen in most chemotherapy recipients with febrile neutropenia (FN), who therefore receive prolonged empiric courses of broad-spectrum antimicrobials (AM). We evaluated the ability of the Karius next-generation sequencing plasma test (KT) to identify infectious etiologies of NF and its impact on AM management.

Methods. This prospective, observational study enrolled 57 patients with ≤500 neutrophils/mm^3. Samples were collected within 24 hours of fever onset (T0) and every 2–3 days. Cell-free plasma DNA was prepared and sequenced in a CLIA/CAP laboratory, human reads excluded, and remaining sequences aligned to a curated pathogen database that includes bacteria, viruses, fungi and parasites. Positive agreement (PA) was defined as KT identification of ≥1 isolate also seen by blood culture (BC). Discordant results were adjudicated by 3 infectious disease specialists as: Definite: KT identified ≥1 organism also seen by MT± 7 days of enrollment; Probable: KT result was a likely cause of NF compatible with clinical diagnosis; Possible: KT result was consistent with an infection but not a common cause of NF.

Results. 56 results (55 subjects) with valid KT and BC results were analyzed. Compared with BC, KT had a PA of 90% (9/10) and negative agreement of 31% (14/45). KT identified >1 organism in 61% (25/41) of the cases. Definite (13), Probable (24) and Possible (4) cases were classified as True Positives. Using clinical adjudication, KT had a sensitivity of 98% (41/42) and specificity of 100% (14/14). The committee would have changed AM therapy 68% (27/40) of the time, had the KT results been available in real-time (~T52–100h). In 8/19 cases (42%) vancomycin would have been discontinued; in 6/27 cases (22%) and in 5/27 cases (19%), anaerobic coverage or antivirals would have been added earlier. Serial analysis of a *Pneumocystis jirovecii* infection indicated that earlier diagnosis and treatment may have prevented morbidity and eventual ICU transfer.

Conclusion. The absence of infectious etiology in NF often leads to broad AM therapy or delay of targeted treatment. Given its sensitivity and ability to detect a breadth of pathogens, the KT can provide useful data for diagnosis and management of NF and may allow for optimization of AM therapy.

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2295. *Streptococcus pneumoniae*-Related Hemolytic Uremic Syndrome (pHUS) and the Identification of Matched Cross Country Serotypes by Plasma Next-Generation Sequencing (NGS)

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Background. Hemolytic uremic syndrome (HUS) describes a clinical presentation of acute kidney injury, microangiopathic hemolytic anemia and thrombocytopenia. Five to 15% of HUS cases are related to *Streptococcus pneumoniae* infection, most often meningitis or pneumonia. Despite the introduction of PCV13 and a decrease in invasive pneumococcal disease in children, the incidence of pneumococcal-related HUS (pHUS) cases is rising for unclear reasons. Efforts to determine whether certain serotypes increase the risk of pHUS are often hampered by negative cultures in patients with suspected pneumococcal disease. Direct microbiologic detection methods, such as next-generation sequencing (NGS), may be useful in identifying pHUS cases. We describe four children with pHUS from two institutions that were identified via NGS of cell-free plasma.

Methods. Four patients with HUS and negative initial cultures were identified. Blood was sent to Karius (Redwood City, CA) for pathogen detection via plasma NGS. Cell-free DNA was extracted and NGS performed. Human sequences were removed and remaining sequences were aligned to a curated pathogen database including over 1000 organisms. Organisms present above a predefined statistical threshold were reported. For serotyping by NGS, sequences were aligned to a collection of 90 serotype-associated *cps* alleles.

Results. All four patients were found be positive for *S. pneumoniae* at extremely high levels (Table 1). Three out of four samples were identified as serotype 3 by NGS and similar to the same strain (*SPN034183*). The fourth sample was consistent with serotype 12A and no strain call was made.

Conclusion. In this case series, we report on four patients with pHUS identified via plasma NGS. These cases demonstrate the potential of NGS for pathogen detection and quantitation in plasma to assist in identification of culture-negative infections, as well as the potential to identify clusters of disease that would likely otherwise have gone undetected.

Table 1: Karius NGS Data

Patient	Immunizations Up to Date	Organism ID	MPM (Molecules/µL)*	Serotype
18 Months (CNMC)	Y	*S. pneumoniae*	1,957,238	3
11 Months (Rady)	Y	*S. pneumoniae*	9,122,698	3
26 Months (Rady)	Y	*S. pneumoniae*	151,941,207	12A
42 Months (Rady)	N	*S. pneumoniae*	1,435,748	3

*Median MPM in non-HUS *S. pneumoniae* positive samples over the last 90 days was 1202 MPM

Disclosures. **S. Venkatasubrahmanyam**, Karius, Inc.: Employee, Salary. **D. Hong**, Karius, Inc.: Employee, Salary.

2296. Development of a Sequencing-Based Assay for Detection of CMV Antiviral Resistance Mutations to Letermovir in UL56

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Background. Antiviral resistance to human cytomegalovirus (CMV) is a growing concern for immunocompromised patients on prolonged antiviral regimens, and CMV remains the most clinically significant infection following allogeneic hematopoietic-cell transplantation. Letermovir targets subunit 2 of the viral terminase complex (UL56) and is approved for CMV prophylaxis in adult stem cell transplant recipients. Resistance to letermovir is conferred by point mutations in the UL56 gene, and with the potential clinical need for antiviral resistance testing, we have developed a UL56 sequencing assay covering 23 identified resistance mutations. Here we summarize the performance characteristics of the UL56 antiviral resistance assay.

Methods. This assay uses automated nucleic acid extraction followed by CMV UL56-specific polymerase chain reaction (PCR). PCR products are subjected to cycle sequencing and capillary electrophoresis, and the resulting sequences are analyzed for the The presence of known resistance mutations between codons 229 and 369 of the UL56 gene. The assay's limit of detection (LOD), precision and accuracy were validated in accordance with accepted regulatory standards using multiple laboratory and clinical CMV strains.