Metagenomic Next-Generation Sequencing for Pathogen Detection and Transcriptomic Analysis in Pediatric Central Nervous System Infections

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Summary: We examined the utility of next-generation sequencing in comparison to usual care in detecting a pathogenic organism in children with central nervous system infections.

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Abstract

Background

Pediatric central nervous system (CNS) infections are potentially life-threatening and may incur significant morbidity. Identifying a pathogen is important, both in terms of guiding therapeutic management, but also in characterizing prognosis. Usual care testing by culture and PCR is often unable to identify a pathogen. We examined the systematic application of metagenomic next-generation sequencing (mNGS) for detecting organisms and transcriptomic analysis of cerebrospinal fluid (CSF) in children with CNS infections.

Methods

We conducted a prospective multi-site study that aimed to enroll all children with a CSF pleocytosis and suspected CNS infection admitted to one of three tertiary pediatric hospitals during the study timeframe. After usual care testing had been performed, the remaining CSF was sent for mNGS and transcriptomic analysis.

Results

We screened 221 and enrolled 70 subjects over a 12-month recruitment period. A putative organism was isolated from CSF in 25 (35.7%) subjects by any diagnostic modality. mNGS of the CSF samples identified a pathogen in 20 (28.6%) subjects, which were also all identified by usual care testing. The median time to result was 38 hours.

Conclusion

Metagenomic sequencing of CSF has the potential to rapidly identify pathogens in children with CNS infections.

Key Words: Next-generation sequencing, metagenomics, meningitis, encephalitis, pediatric
Background

Pediatric central nervous system (CNS) infections are potentially life-threatening. Mortality and morbidity occur in up to 28% and 56% of patients respectively [1,2]. CNS infections encompass a range of manifestations that vary based on pathogen [3]. Identifying a pathogen is vital, both in terms of guiding therapy, but also in characterizing prognosis. Culture and polymerase chain reaction (PCR) assays are frequently unable to identify a pathogen. For encephalitis, in over 40% of patients, usual care testing is unable to identify a pathogen [3–5]. In bacterial meningitis, culture may be negative after antibiotic pretreatment, a common occurrence [2,6]. PCR offers rapid and sensitive testing, but is restricted to the pathogens for which the platform is targeted. Given the limitations of available diagnostics, “Pan-omic” platforms offer promise [7,8]. One such modality is metagenomic next-generation sequencing (mNGS) for pathogen DNA and RNA [9,10]. This methodology poses the opportunity to broadly evaluate for pathogens by testing a single specimen. Turn-around times have shortened, making mNGS techniques more applicable as a method of achieving a timely diagnosis. We conducted a prospective, multi-site, study to evaluate DNA and RNA sequencing of cerebrospinal fluid (CSF) for identification of pathogens in children with a suspected CNS infection.

Methods

Trial design and oversight:

Pediatric Infectious Disease Precision Medicine Using Sequencing Evaluation of CSF (PIPSEC, NCT03796546) was a prospective, multi-site study to evaluate DNA and RNA sequencing of CSF for identification of pathogens in subjects who had undergone evaluation for a CNS infection. The members of the research team had final responsibility for the trial design and oversight.

Patient Consent Statement

Written consent was obtained from subjects or their guardian. The Western Institutional Review Board and the Children’s Hospital of Orange County Institutional Review Board provided human subject protection oversight.

Eligibility Criteria:

Eligible subjects were less than 18 years of age undergoing evaluation for CNS infection and found to have a CSF pleocytosis. Suspicion for CNS infection was determined based on the clinical impression of the ordering provider. CSF pleocytosis was defined as a white blood cell count (WBC) > 15 cells/µL with <5000 cells/µL red blood cells (RBCs) in the same sample [11]. A protocol deviation was approved for 2 subjects with 14
cells/μL WBC’s in the CSF at the request of the study site principle investigator (PI).

Study Outcomes:
The primary aim of this study was to investigate the diagnosis rate of mNGS for pathogen detection in CSF samples compared to usual care testing performed on CSF samples from the same subject [12]. Usual care testing consisted of all CSF diagnostic testing performed on the case in a CAP/CLIA certified laboratory (see supplement Table 1). Usual care testing was done at the discretion of the clinical team and thus varied by subject. Data was extracted from the electronic medical record (EMR). Site PI’s were additionally sent clinician surveys to assess clinical utility of mNGS results in relation to usual care. The treating physician, site PI’s, and study PI then reviewed the CSF mNGS results in comparison to usual care testing from CSF and clinical presentation to determine the likelihood that the identified organism was indeed putative. Each mNGS result was identified as a true positive (TP), false positive (FP), true negative (TN), or false negative (FN) relative to usual care testing from CSF (composite of PCR, culture, and other CSF testing) as the reference standard. Determination of TP or TN was made when mNGS and usual care testing were concordant. In the event of discordant results, mNGS was compared to the reference standard composite of usual care testing. If mNGS identified an organism that was not identified by usual care testing, this was considered a FP. If mNGS failed to identify the organism identified by usual care testing, this was considered a FN. The p values for comparison of means were calculated using the Student t test.

Sequencing:
CSF samples were collected from the laboratory after usual care testing had been performed. The minimum volume for mNGS testing was 0.5ml. Samples for mNGS testing were stored at -80C until they were shipped to the reference laboratory. Samples were shipped via express courier to achieve a less than 24 hour transit time. CSF samples were tested with research use only next-generation shotgun DNA and RNA sequencing protocols; the resulting data were analyzed with the Explify Platform (IDbyDNA Inc, Salt Lake City, UT) as previously described [10,13,14]. DNA (after host depletion) and RNA were extracted separately from residual samples. DNA and RNA sequencing libraries were prepared with the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA) and sequenced on a NextSeq550 instrument (Illumina, San Diego, CA) using mid-output kits. In this study, a range of 8-18 total libraries, including external controls, were sequenced per run for a median depth of 9.98 x 10^{6} and 1.1 x 10^{7} single-end, 150-bp sequencing reads per sample-derived DNA and RNA library, respectively. Sequencing reads were adapter-trimmed and quality-filtered as part of the Explify analysis.
The assay was quality controlled using internal control organisms spiked into each sample at the lysis step and the inclusion of three external controls (positive, negative, and blank) in each sample batch. Sample results were released after performance metrics for internal and external controls were evaluated for data quality and quantity. Sequencing data analysis was performed with the Explify platform. RNA-seq and DNA-seq data were analyzed together and the final result for a given sample was based on evidence from either.

Transcriptomic analysis:

Host transcriptomic counts were analyzed using Rosalind (OnRamp Bioinformatics, San Diego, CA). Sample transcript counts were generated with Kallisto (v.0.46.0) normalized by relative log expression using DESeq2 R library [15]. Heatmaps generation and clustering were performed using the Partitioning Around Medoids methods using the fpc R library [16]. Differential gene expression (DEG) was considered as a fold change of more than 1.5 and a false discovery rate (FDR) cutoff of 0.05 [17]. Hypergeometric distribution was utilized to analyze pathway enrichment, gene ontology, domain structure, and other ontologies. The topGO R library was used to determine local similarities and dependencies between gene ontology terms to perform Elim pruning correction [18]. Several databases were utilized for enrichment analysis including REACTOME, HOMER, and molecular signatures database (MSigDB) [19–21]. Weighted correlation network analysis (WGCNA) analysis was performed using filtered log2 transformed RNA-seq data [22]. This was applied to construct scale-free networks that specify gene modules [22]. To explore the modular structures of the co-expression network, the adjacency matrix was transformed into a topological overlap matrix [22]. WGCNA parameters were as follows: minimum module size equal to 30 genes, tree-cut height set to 0.99. Significant modules were defined as those with a strong correlation (r > 0.4) and a p-value of less than 0.05. Interacting genes were visualized as a network using String version 11.0 (http://string-db.org). K-means clustering was used to determine gene clusters of interest and/or highly connected genes within this isolated M25 network [23]. mNGS data were pseudo-aligned to the human genome (assembly hg38) using Kallisto to yield host response transcriptomic data and then filtered for rRNA reads prior to downstream analysis. The sequencing depth for mapped reads varied from 30,000 reads to 5 million reads (mean = 1.5 million).

Results

We screened 221 subjects at 3 study sites from January 2019 to January 2020 and enrolled 70 subjects (Supplement Figure 1). See Table 1 for demographic information. mNGS of the CSF samples identified a pathogen in 24 subjects. Twenty of these were deemed to be putative (TP) versus 25 by usual care testing of
CSF (Table 2 and Table 3). Two subjects, one with acute disseminated encephalomyelitis and another with autoimmune encephalitis, received a non-infectious diagnosis. Nine subjects had enterovirus meningitis.

Discordant results that the treating physician deemed to be FP results by mNGS were identified in 4 subjects. The organisms identified for these subjects included Cryptococcus spp, Staphylococcus warneri, Mucor circinelloides, and Streptococcus bovis. In these 4 cases, usual care testing, including gold standard CSF culture, did not identify an organism, and the treating clinician did not deem the mNGS result to be clinically relevant.

In 5 subjects, CSF mNGS was deemed falsely negative with relation to usual care CSF testing. Two involved an infected ventriculoperitoneal shunt, one due to Staphylococcus epidermidis and the other to Staphylococcus aureus (both identified on culture). Upon chart review, the site of CSF sampling was not clear for either case (the sample may have been obtained proximally or distally from the shunt). Upon review of the mNGS data, both organisms were detected, but did not meet pre-specified thresholds for reporting. In the third case, PCR for Epstein-barr virus (EBV) was positive from the CSF at just 151 IU/L. The subject improved without antiviral therapy. In the fourth case, CSF culture from a subject with chronic meningocencephalitis recovered a single colony of Cryptococcus neoformans and CSF cryptococcal antigen was positive. A prolonged antifungal course resulted in a full recovery. In the fifth case, human herpes virus 6 (HHV-6) was identified on a CSF multiplex PCR panel. This patient had full clinical recovery and antiviral therapy was not initiated. Of note, the HHV-6 target from the multiplex PCR panel was positive for 2 other subjects. In one subject, described above (Subject 2016), the CSF culture was also positive for Staphylococcus aureus and was accordingly adjudicated as an mNGS false negative. In the other subject (Subject 1004), a dedicated HHV-6 PCR was negative, and this subject was adjudicated as “concordant CSF mNGS and CSF usual care” and therefore an mNGS TN. Dedicated HHV-6 PCR was not sent for any other subjects.

CSF culture identified a putative organism in 12 cases (17.1%). A CSF PCR multiplex panel was utilized for 51 subjects, 15 (29.4%) of which were positive. In one subject with a negative culture and multiplex PCR panel, 16S broad-range PCR and mNGS both detected Streptococcus dysgalactiae. Median time to result from the time sample was received for CSF mNGS was 38.0 hours (IQR 32.6-60.5 hours). The mean CSF nucleated cells was higher in the subjects for whom a pathogen was detected by mNGS in comparison to subjects for whom no organism was detected (1499.6 cell/ul vs 410.7 cells/ul, p value 0.05). Mean CSF protein value (228.3mg/dL vs 184.4mg/dL, p value 0.02) and mean glucose level (38.2mg/dL vs 52.6 mg/dL, p value 0.004) were also both higher in subjects for whom an organism was detected by mNGS versus subjects for whom no organism was detected. Based on survey results, clinicians indicated that CSF mNGS helped in the management in 17.1% of
cases. In ten of these cases, the negative mNGS results was used to buttress the clinical impression that a treatable infectious etiology was not missed by CSF testing. In one case, the clinician explicitly stated that the mNGS result was used to provide clearance for surgical intervention. In another case, the clinician was reassured by concordance between the positive mNGS and usual care result both identifying the same viral pathogen.

Transcriptome analysis:

In addition to pathogen data, mNGS also generated incidental host transcriptomic data. Data were compared for all subjects using multidimensional scaling (MDS) and did not globally cluster by diagnosis gender, age, CSF white blood cell count, or neutrophil predominance (Supplemental Figure 2A). A comparison of subjects diagnosed with viral (N = 12) as compared to bacterial (N = 11) meningitis identified 409 differentially expressed genes (DEGs) (Figure 1) and overall clustered by infectious diagnosis. DEGs included up-regulation of genes identified in host response to viral and bacterial infection, including interleukin 1A/B and interleukin 8 in bacterial meningitis. Gene ontology analysis identified pathways that included immune response and both leukocyte and neutrophil activation (Supplemental Figure 2). Interleukin 1B (p-adj = 0.004) and CXCL8 (p-adj = 0.0002) were most associated with bacterial meningitis, and CCL8 (p-adj = 0.007) with viral meningitis as compared to all other patient samples (Figure 1C). WGCNA pathway analysis identified a statistically significant module of 68 co-expressed genes (Dark red module, Supplementary Figure 3). Quantitative trait analysis was not significant for any other patient features. Taken together, gene ontology and WGCNA analysis suggest interleukin 1B may be a key regulator of the proinflammatory response observed in bacterial meningitis (Supplemental Figure 4). Diagnostic performance was assessed using Receiver Operator Characteristic (ROC) curves with a corresponding area under the curve (AUC) of 0.95 (95% confidence CI 0.89-0.998) for IL1B (Figure 1D). A sub-analysis of the FN mNGS results suggested infection based on host response in 2 of these 4 patients.

Post Hoc Cost Analysis:

Median cost of usual care testing was $531.09 (IQR $439.00-$721.09). An average of 3 usual care CSF tests (including culture) were sent per subject. Per-sample direct cost of CSF mNGS laboratory testing is dependent on the number of samples being run at a time and is estimated to range between $390.00 and $2000.00. In this study, no more than 5 CSF samples were sequenced per run to expedite the turn-around time. Per-sample direct cost for shotgun sequencing can be decreased by an order of magnitude through optimization of the balance...
between analytical sensitivity, test volume, and turnaround time considerations.

Discussion

We describe application of metagenomics in the diagnosis of pediatric CNS infections that included all consenting subjects meeting enrollment criteria rather than only a referred subset. As has been previously described, an infectious etiology was not identified for most subjects with suspected CNS infection [5]. A putative organism was isolated from CSF in 25 (35.7%) subjects, and of these, mNGS of the CSF samples identified a pathogen in 20 (28.6%) subjects (Table 3). Using the above described adjudication scheme, FP results by mNGS were identified in 4 subjects, and in these subjects, usual care testing did not identify a pathogen. In 5 cases, a putative organism was recovered by usual care testing of the CSF, but not by CSF mNGS. Two of these cases involved hardware infection, and in another subject with chronic meningoencephalitis, a single colony of Cryptococcus neoformans was isolated from CSF. In the fourth subject, although EBV was detected by PCR, the viral load was just 151 IU/L. The 5th case was a positive result for HHV-6 identified by CSF PCR multiplex panel, which is of unclear clinical significance (previous studies have described FP results for this target) [24]. In these cases, burden of organism was likely low even prior to initiation of antimicrobial therapy, which may indicate that, for some organisms and in certain circumstances, culture and PCR remain more sensitive [8]. Upon review of the subjects with hardware infections (Subjects 2016 and 3014) deemed as mNGS FN’s, a signal for S. aureus and S. epidermidis was detected but below the reporting threshold (Supplement Figures 5 and 6). Limitations in the application of this study, including imperfect sampling, may have further degraded the sensitivity of mNGS.

CSF sampling is important for management of CNS infections. Sensitivity of culture in bacterial meningitis is high, but diminished if CSF is obtained after initiation of antibiotics [6]. Culture is less sensitive for fungi and mycobacteria. PCR may demonstrate high sensitivity for certain targets, but requires clinicians to suspect the organism prior to testing, which may lead to missed diagnoses and to broad and potentially unnecessary testing. mNGS may be used to evaluate for the presence of many pathogens with a single test. Wilson et al examined use of mNGS in 204 subjects with suspected CNS infections [9]. Among 58 subjects in whom an organism was identified, mNGS identified 13 (22%) that were not identified by usual care testing. A notable difference in this cohort from our study is the older study population, many of whom were immunocompromised. Additionally, many of these subjects were recruited after an extensive evaluation, increasing the likelihood that mNGS would recover an uncommon pathogen where usual care had already failed to discover an etiology. The subjects
recruited in our study were more often previously healthy, and therefore more likely to have infection with common pathogens. mNGS was additionally used as a first-tier test in conjunction with usual care. This decreased the likelihood that mNGS would detect an organism not identified by usual care testing. In a cohort more similar to our study, Hong et al. evaluated mNGS for CNS infections in a resource-limited setting, and found that mNGS of the CSF identified a pathogen in 14/19 CSF samples that were positive by PCR [25]. Using PCR as the reference assay, they calculated a sensitivity and specificity of 74% and 66% respectively. Our data shows that mNGS offers similar diagnostic yield, though in order to fully understand the limit of detection and the importance for clinical care, larger studies will be needed, especially as mNGS techniques involving enrichment and depletion technologies are refined. mNGS may be better utilized as an adjunct to current usual care testing, especially when usual care testing has already failed to recover a pathogen. In addition, while cost has been a limiting factor in the adoption of mNGS, if mNGS is ultimately shown to provide similar results to usual care in a shorter time at a similar price point, mNGS may prove to be more attractive [26]. Turnaround time can additionally be improved by local implementation of mNGS testing, a model which has become more common in tertiary care centers.

Transcriptome analysis was utilized to evaluate host expression of genes associated with the immunologic response. Subjects with bacterial meningitis were found to have a distinct pattern of gene expression compared to those with a viral etiology. Notably, CXCL8, CXCL1, TNF, and interleukin 1A/B have been found to be expressed in bacterial meningitis [27-29]. Conversely, CCL8 has been found to be associated with viral infections [27,30,31]. The results of our analysis show increased expression of CXCL8, CXCL1, and TNF in the subjects with a bacterial infection and increased expression of both CCL8 and IFI6 in subjects with viral infection (Figure 1). WGCNA analysis independently identified interleukin 1B as a hub gene in the host response to bacterial meningitis, suggesting it may regulate proinflammatory gene expression. Interleukin 1 genotype has been implicated as contributing to the risk of fatal meningococcal meningitis [32]. The clinical utility of these findings needs to be clarified with further studies, but potentially, host response transcriptome analysis may prove useful in characterizing inflammatory response associated with specific pathogens and in attributing pathogenicity when an organism is identified [28,33-37]. Furthermore, characterizing host response may additionally inform the differential diagnosis when an organism is not found.

This study has several limitations. The epidemiology of CNS infections may reflect geographic distribution, limiting generalizability. The number of enrollees is additionally likely too small to adequately capture rarer
pathogens associated with CNS infections. Adjudication of results as putative or not with respect to a composite of usual care tests used to create a reference standard was hampered by the variability in the types of testing sent. While every subject had CSF culture sent, the choice to use serologic or molecular based assays was purely at the discretion of the clinical team, and the validity of comparing mNGS results to a standard reference that is not uniform throughout the cohort is limited. Additionally, characterization of mNGS results as FP are based on published epidemiology of CNS infection in children, which are biased toward historic norms and limited by the sensitivity of usual care testing modalities (including CSF culture). We additionally were unable to utilize adjunctive orthogonal testing such as targeted or broad range PCR due to unavailability of additional CSF sample. FP mNGS results may be attributable to sample misidentification, contamination introduced during sample collection or processing, biases within reference databases, or the analysis tools used. For the determination of a TN specifically, by using the criteria of no organism detected by any testing method, we may have incorrectly adjudicated subjects with organisms that were either in low abundance or difficult to isolate. Additionally, subjects with suspected infection may instead have autoinflammatory or autoimmune phenomenon that may be challenging to differentiate from an infection [3,9,38,39]. CSF parameters vary based on age, and the low cutoff used in the inclusion criteria may have allowed for enrollment of some subjects with physiologically normal CSF [11,40]. Conversely, more indolent CNS infection may not generate a significant CSF pleocytosis. Lastly, the protocol stipulated that only residual CSF be used for mNGS, which likely limited diagnostic yield, especially in cases where burden of organism was lower [8].

Conclusion

In this prospective study of pediatric CNS infections, mNGS of the CSF identified an organism in 28.6% of subjects. While certain usual care tests remain more sensitive for some targets, mNGS may have value as an adjunctive diagnostic tool, especially in situations where standard testing is known to have limited yield. Furthermore, this is specific to the sequencing and analysis methods used in this study, as sensitivity and specificity of mNGS varies depending on choice of wet lab methodology and analysis tools. Further studies are required to clarify the best use of mNGS in the evaluation of pediatric CNS infections.
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Conflicts of Interest:

Robert Schlaberg is the Chief Medical Officer of IDbyDNA. Lauge Farnaes is the Director of Medical & Scientific Affairs at IDbyDNA. Benjamin Briggs is the Associate Director of Medical & Scientific Affairs at IDbyDNA. Rita Stinnett is the Associate Director of Clinical Studies at IDbyDNA. Toni Schwarz is a scientist with IDbyDNA’s Medical and Scientific Affairs department. Heng Xie is the Associate Director of Laboratory Technology at IDbyDNA. IDbyDNA sponsored this study.
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| Characteristic                          | Value          |
|----------------------------------------|----------------|
| Male Sex, n (%)                        | 40 (57.1)      |
| Age (years), median (IQR)              | 3.8 (0.2-11.8) |
| Rady Children's Hospital San Diego, CA, n (%) | 31 (44.3) |
| Children's Hospital Orange County, CA, n (%) | 24 (34.3) |
| Nicklaus Children's Hospital, FL, n (%) | 15 (21.4)      |
| Immunocompromised, n (%)               | 4 (5.7)        |
| Hispanic ethnicity, n (%)              | 41 (58.6)      |
| Caucasian/White, n (%)                 | 46 (65.7)      |
| African-American/Black, n (%)          | 2 (2.9)        |
| Asian, n (%)                           | 2 (2.9)        |
| Other, n (%)                           | 12 (17.1)      |

**Presenting Symptoms**

| Symptom                  | Value          |
|--------------------------|----------------|
| Fever, n (%)             | 51 (72.9)      |
| Vomiting, n (%)          | 28 (40.0)      |
| Seizures, n (%)          | 16 (22.9)      |
| Lethargy, n (%)          | 30 (42.9)      |
| Altered Mental Status, n (%) | 26 (37.1) |
| VP shunt, n (%)          | 6 (8.6)        |
| Received antibiotics prior, n (%) | 28 (40.0) |

**CSF Parameters**

| Parameter                  | Value          |
|----------------------------|----------------|
| Nucleated cells/μL, median (IQR) | 109 (35.5-513.5) |
| Erythrocytes cells/μL, median (IQR) | 16 (60-368.0) |
| Protein mg/dL, median (IQR) | 97 (41.8-168.0) |
| Glucose mg/dL, median (IQR)   | 47.5 (40.0-56.0) |
| Length of Stay, median days (IQR) | 6 (3.0-18.5) |
| Admitted to ICU, n(%)       | 37 (52.9)      |
| Death, n (%)               | 3 (4.3)        |
| Total Patients             | 70             |

Table 1. Research subject demographic characteristics
Table 2. Comparison of subjects for whom CSF testing was positive by any method.

| Subject | CSF culture | HSV PCR | EV PCR | CSF multiplex PCR | CSF other test (positive result) | CSF mNGS | total sequencing reads | number of reads for identified organism |
|---------|-------------|---------|--------|-------------------|-------------------------------|----------|----------------------|----------------------------------------|
| 1001    | Haemophilus influenzae | negative | not done | not done | Haemophilus influenzae | 2.94E+07 | 5.17E+04 |
| 1007    | Enterococcus faecalis | negative | not done | not done | Enterococcus faecalis | 2.28E+07 | 2.94E+04 |
| 1015    | Staphylococcus epidermidis | not done | not done | not done | Staphylococcus epidermidis | 2.18E+07 | 5.64E+04 |
| 1025    | Enterovirus | not done | negative | positive | Enterovirus | 2.54E+07 | 2.26E+04 |
| 1027*   | no organisms | negative | not done | positive | not done | Enterovirus | 1.67E+07 | 5.17E+04 |
| 1028    | no organisms | negative | not done | positive | not done | Enterovirus | 1.66E+07 | 1.30E+01 |
| 2001    | Streptococcus agalactiae | not done | not done | not done | Streptococcus agalactiae | 2.26E+07 | 1.58E+05 |
| 2005    | Streptococcus pneumoniae | not done | negative | positive | Streptococcus pneumoniae | 3.17E+07 | 5.73E+05 |
| 2008    | Staphylococcus aureus | negative | positive | not done | Staphylococcus aureus | 2.03E+07 | 3.06E+03 |
| 2010    | Staphylococcus aureus | not done | not done | positive | Staphylococcus aureus | 2.38E+07 | 3.53E+03 |
| 2011    | no organisms | negative | not done | positive | not done | no pathogens detected | 2.37E+07 | - |
| 2015    | no organisms | not done | not done | not done | Staphylococcus aureus | 1.33E+07 | - |
| 2016    | Staphylococcus aureus | not done | not done | not done | Staphylococcus aureus | 1.83E+07 | - |
| 3010    | no organisms | not done | not done | not done | negative | no pathogens detected | 1.85E+07 | - |
| 3014    | Staphylococcus epidermidis | not done | not done | not done | Staphylococcus epidermidis | 1.25E+07 | - |

Note: Two unique tubes of residual CSF were received for 1027. Each was processed and analyzed separately by the reference lab; both samples produced the same result. The read counts listed in the table represent the mean sequencing reads for these two samples.
| CSF mNGS % positivity | 28.6% |
|------------------------|-------|
| CSF mNGS positive predictive agreement | 80.0 (95%, 59.3-93.2%) |
| CSF mNGS negative predictive agreement | 91.1 (95%, 78.8-97.5%) |
| % agreement (kappa)     | 87.1 (0.80) |

Table 3. Evaluation of a metagenomic assay for detection of CSF pathogens against a composite reference of usual care testing. Each mNGS result was identified as a true positive (TP), false positive (FP), true negative (TN), or false negative (FN) relative to usual care testing from CSF (composite of PCR, culture, and serologic testing for each subject) as the reference standard. A culture was sent for every subject. Dedicated enterovirus PCR was sent for 19 subjects, and dedicated HSV PCR was sent for 13 subjects. A multiplex PCR was sent for 52 subjects. A dedicated EBV PCR was sent for 1 subject. Broad range universal PCR was sent for 1 subject.
Figure 1. A. A heat map comparing PIPSEC subjects with bacterial and viral diagnoses identified 409 differentially expressed genes with FDR > 0.05 and fold change ≥ 1.5. B. Volcano plot identifying differentially expressed genes between bacterial and viral meningitis. C. Scatter plot depicting mean, SEM, and individual values. ** p-adj < 0.01, *** p-adj < 0.0001 as compared to all other groups with a fold change of 10.0 for IL1B and 18.0 for CXCL8 in bacterial meningitis, and 14.0 for CCL8 in viral meningitis as compared to all other samples. D. ROC for IL1B and CCL8. AUC = Area Under Curve.