Evaluation of the FilmArray™ for Rapid Pathogen Identification from Cerebrospinal Fluid (CSF) in Children

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Background

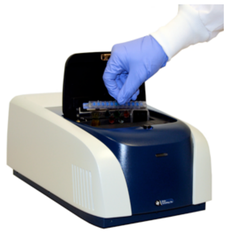
Meningitis is a severe infection that can cause significant morbidity and mortality. Rapid etiologic diagnosis is critical to the initiation of appropriate therapy, however conventional testing can require up to 48 hours to identify the causative agent. Cultures are frequently negative, often due to antibiotic pre-treatment prior to CSF sampling. A molecular system that can rapidly identify bacteria and viruses causing meningitis could improve the medical management of this infection.

Materials and Methods

The FilmArray™ (FA; BioFire Diagnostics, LLC, Salt Lake City, UT) performs automated nucleic acid purification and multiplex PCR to identify bacterial and viral pathogens directly from clinical specimens. The FA Meningitis/Encephalitis Panel (FA ME) has been developed to detect and identify 17 bacterial, viral, and fungal pathogens causing meningitis in adults and children. We used a research use only version of the panel to perform a retrospective study of archived CSF samples from 118 children <18 years presenting to Primary Children's Hospital with concern for meningitis. Conventional CSF testing (bacterial culture and/or viral testing) was ordered at the discretion of the treating physician. FA studies were performed at the University of Utah. FA ME results were compared to conventional testing.

Figure 1. The FilmArray™ Instrument and Pouch

The FilmArray is a medium-scale fluid manipulation system which performs high-order nested multiplex PCR in self-contained, disposable, thin-film plastic pouch. The FilmArray processes a single sample, from nucleic acid purification to result, in a fully automated fashion in ~1 hour. The FilmArray Meningitis / Encephalitis (FA ME) Panel requires 200 µL of CSF for testing.



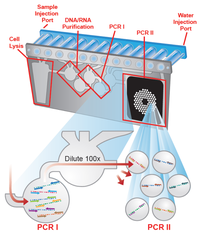


Table 1. FilmArray™ Meningitis/Encephalitis Panel

Bacteria	Viruses	Viruses cont.	Fungus
Escherichia coli K1	Cytomegalovirus (CMV)	Parechovirus	*Cryptococcus neoformans/gattii*
Haemophilus influenzae	Enterovirus	Varicella-zoster virus (VZV)	
Listeria monocytogenes	Epstein-Barr virus (EBV)		
Neisseria meningitidis	Herpes simplex virus 1 (HSV-1)		
Streptococcus agalactiae	Herpes simplex virus 2 (HSV-2)		
Streptococcus pneumoniae	Human herpesvirus 6 (HHV-6)		

Table 2. Demographic and Clinical Characteristics of Enrolled Children (n=118)

Variable	Number (% or range)
Demographics	
Age, months, median	2 (0-215)
Male	65 (55%)
Clinical Characteristics	
LOS days, median	3 (1-200)
Hospitalization in ICU	41 (34%)
Death	1 (1%)
Pathogen Detection	
Conventional testing	
Bacterial pathogen	18 (15%)
True bacterial pathogen*	11 (9%)
Viral pathogen	4 (3%)
Negative	96 (81%)
FilmArray	
Bacterial pathogen	14 (13%)
True bacterial pathogen*	14 (13%)
Viral pathogen	14 (13%)
Negative	94 (80%)
Laboratory Testing	
CSF WBC, median (range)	4 (1-15750)

* A true bacterial pathogen was defined as a pathogen commonly associated with meningitis/encephalitis in a patient without intracranial hardware. Pathogens associated with shunt infections were excluded as shunt samples were not included in the study

Results Summary

- 18 children had positive CSF cultures, of which 11 grew true ME pathogens
- Nine of the eleven were included on the FA ME panel
- FA detected 8/9 cultured panel bacteria, and 6 additional bacterial pathogens
- The median CSF WBC count in children with positive bacterial cultures was 438
- Median CSF WBC for children with positive bacterial testing by FA ME was 1749
- The difference in mean CSF WBC could be accounted for by low CSF WBC in samples culture-positive for presumed contaminants
- Only 4 children had viruses detected by conventional methods, including PCR of blood or CSF. Many children did not have viral testing performed
- FA ME detected viruses in 14 samples, including 4 also positive for true pathogenic bacteria (*S. pneumoniae* (1)*; E. coli* (1); *S. agalactiae* (1) and *N. meningitidis* (1))
- EBV was detected most often as a co-pathogen; HHV-6 more often alone

Table 3. Identification of Pathogens from CSF

Organism	No. identified by conventional testing	No. identified by FilmArray
S. pneumoniae	3	4
S. agalactiae	1[1]	1
E. coli	1	3
H. influenzae	3	4
N. meningitidis	1	1
Enterovirus	2[2]	2
Epstein-Barr virus	1	5
Human herpes virus 6	1[3]	6
Herpes simplex virus 1	0[4]	1
Parechovirus	None tested	1
No pathogen detected	96	94
Not on panel	7[5]	0

1: The FA detected one *S. agalactiae* not detected by culture, but missed one which was detected by culture. 2: The FA detected one enterovirus not detected by conventional testing but also missed one. 3: HHV6 detected only in blood; CSF not tested and negative by FA. 4: Sample positive by FA was not sent for HSV-1 conventional testing. 5: Pathogens not on panel included *C. koseri, K. pneumoniae,* coagulase-negative staphylococci and viridans streptococci.

Acknowledgments: BioFire Diagnostics donated the RUO reagents and FilmArray™ instrument

Table 4. Clinical and Laboratory Data by Detection Method

Pathogen Detected (n)	Median (Range) CSF WBC	Outcome (ICU/Death)
Conventional testing		
Bacterial pathogen (18)	438 (2-15750)	12/1
True bacterial pathogen (11)	3013 (4-15750)	8/1
Viral pathogen (4)	2 (1-6)	0/0
No pathogen (96)	3 (1-13063)	30/0
FilmArray		
Bacterial pathogen (14)	1749 (2-15750)	12/0
True bacterial pathogen (14)	1749 (2-15750)	12/0
Viral pathogen (14)	9 (1-15750)	5/0
Viral pathogen alone* (10)	5 (1-8892)	2/0
No pathogen (94)	3 (1-6025)	28/1

* Viral detection without true bacterial co-detection

Table 5. Clinical and Lab Data: Pathogens Detected by FA Only

Pathogen Detected (n)	CSF WBC Median (Range)	Outcome (ICU/Death)
Bacterial Detections (6)	1398 (2-13063)	4/0
H. influenzae (1)	2790	1
S. pneumoniae (1)	13063	1
S. agalactiae (2)	6; 3775	2
E. coli (2)	2; 2	0
Viral Detections (12)	14 (1-8892)	5/0
EBV (4)	431 (2-15750)	2
EBV without bacterial detection (1*)	154	0
HHV6 (6)	14 (1-8892)	3
HHV6 alone (5)	11 (1-8892)	2
HSV (1)	1	0
EV/Parechovirus (1*/1)	154/1	0

*EBV/EV co-detection

Conclusions

- The FilmArray Meningitis/Encephalitis Panel is a sensitive tool for the rapid identification of true pathogens from CSF and may identify causative pathogens not detected by conventional testing
- More than twice as many viruses were detected by FilmArray when compared to conventional testing, particularly HHV-6 and EBV. Often, no viral testing was done
- Further study of the clinical significance of HHV-6 and EBV detection is warranted
- Rapid detection of pathogens from CSF using FilmArray has the potential to improve treatment and outcomes for patients with meningitis