Comprehensive Testing of CSF Specimens Using The FilmArray® ME Panel Identifies Viral Infections Overlooked Using Current Clinical Practices

#1668

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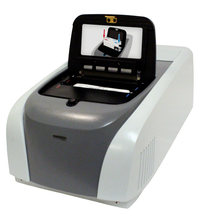
INTRODUCTION/BACKGROUND

Infections of the central nervous system (CNS), such as meningitis and encephalitis, are potentially life-threatening. Caused by a myriad of pathogens (bacteria, viruses, fungi), CNS infections often present with similar clinical symptoms. Accurate diagnosis is limited because current methods for pathogen detection in cerebrospinal fluid (CSF), such as culture or single PCR reactions, have a long time-to-result and do not provide a complete answer. This may lead to additional patient health risk and rising healthcare costs. Rapid, comprehensive testing for the most common causes of CNS infections has the potential to change the approach to patient therapy while leading to healthcare cost savings.

To address this unmet diagnostic need, BioFire Diagnostics, LLC developed the FilmArray® Meningitis/Encephalitis (ME) Panel for use on the FilmArray System. The FilmArray ME Panel simultaneously tests for six bacteria, eight viruses, and two fungi using approximately 200 µL CSF. Two minutes of user hands-on time are required, and a comprehensive result is returned in about one hour.

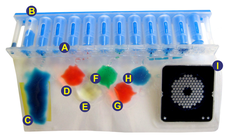
The FilmArray System

The FilmArray is a lab-in-a-pouch medium-scale fluid manipulation test performed in a self-contained, disposable, thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion.



The FilmArray ME pouch has a fitment (B) containing freeze-dried reagents and plungers that plunge liquids to the film portion of the pouch. This portion consists of stations for cell lysis (C), magnetic-bead based nucleic acid purification (D & E), first-stage multiplex PCR (F & G) and an array of 102, second-stage nested PCRs (I).

PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second-stage PCR product is detected in a melting analysis using a fluorescent double-stranded DNA binding dye, LCGreen®.



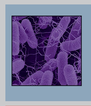
A. Fitment with freeze-dried reagents
B. Plungers- deliver reagents to blisters
C. Sample lysis and bead collection
D. Wash station
E. Magnetic bead collection blister
F. Elution Station
G. Multiplex Outer PCR blister
H. Dilution blister
I. Inner Nested PCR array

MATERIALS AND METHODS

Following institutional review board (IRB) approval, for use of de-identified, discarded cerebral spinal fluid specimens, a pilot evaluation of the FilmArray ME Panel was conducted using 178 frozen CSF samples collected at Loyola University Medical Center (LUMC) from September 2013 through April 2014. The specimen set was composed of residual CSF that had been submitted to LUMC by clinicians for investigation of pathogens due to suspicion of CNS illness. Specimens were tested with the FilmArray ME Panel and the results were compared to bacterial culture, cryptococcal antigen testing, and viral PCR testing that had been performed based on clinician test requests at the time of specimen submission. Any discrepancies between the FilmArray panel and clinical results were investigated with additional independent PCR tests followed by bi-directional sequencing.

THE FILMARRAY MENINGITIS/ENCEPHALITIS (ME) PANEL

Simultaneous detection of 15 targets:



Bacteria

- *Escherichia coli* K1
- *Haemophilus influenzae*
- *Listeria monocytogenes*
- *Neisseria meningitidis*
- *Streptococcus agalactiae*
- *Streptococcus pneumoniae*

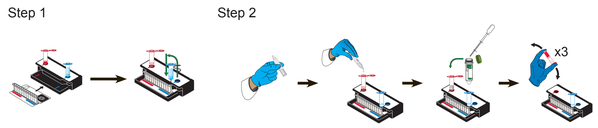
Fungi

- *Cryptococcus gattii/neoformans*

Viruses

- Cytomegalovirus
- Enterovirus
- Epstein-Barr virus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6
- Human parechovirus
- Varicella zoster virus

Sample Processing and Pouch Loading Instruction



Testing requires minimal pre-processing of specimens. CSF and FA Sample Buffer are combined in a novel filter-injection vial and then loaded into the FilmArray ME pouch. The user enters the sample and pouch type (using a barcode reader) into the software and initiates a run.

RESULTS

LUMC laboratory methods collectively identified five pathogens in the 178 samples consisting of Enterovirus (4) and VZV (1). In contrast, the comprehensive FilmArray ME Panel detected 14 pathogens consisting of Enterovirus (6), HHV6 (4), EBV (1), Enterovirus + EBV (1) and VZV (1). The FilmArray ME Panel was concordant with all LUMC results. Secondary PCR assays were in agreement with the FilmArray ME Panel in 12 of the14 specimens: one EBV and one HHV6 detection could not be confirmed.

Figure 1. Age and Sex Breakdown of Patient Samples Tested

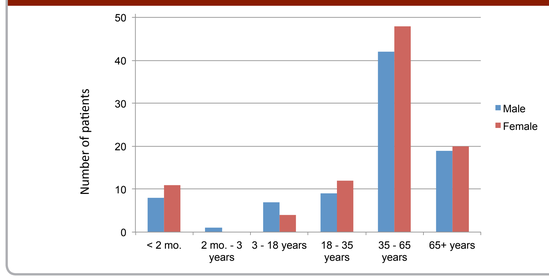


Figure 2. Venn Diagram of Physician Ordered Tests

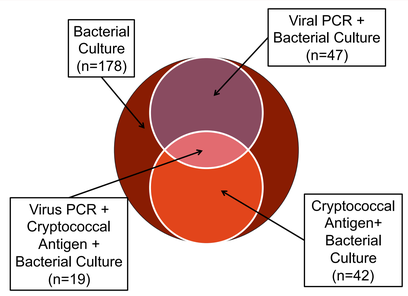


Figure 3. Physician Ordered Virus PCR Tests Performed at LUMC

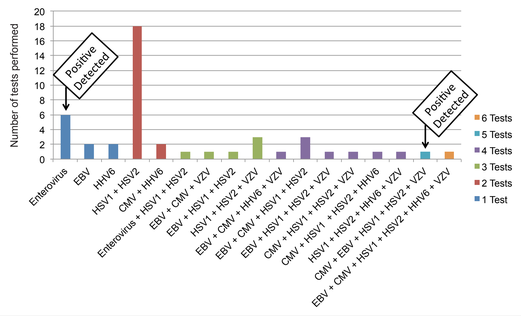


Table 1. Pathogen Detections in CSF with Ordered Test or The FilmArray ME Panel

Pathogen	LUMC (n=178)		FilmArray ME (n=178)
	Ordered Test	Detected	Detection
Cryptococcus gattii/neoformans	42	0	0
CMV	12	0	0
Enterovirus	7	4	7
EBV	12	0	2
E. coli K1	178	0	0
H. influenzae	178	0	0
HSV1	33	0	0
HSV2	33	0	0
HHV-6	9	0	4
Parechovirus	N.T.	N.T.	0
L. monocytogenes	178	0	0
N. meningitidis	178	0	0
S. agalactiae	178	0	0
S. pneumoniae	178	0	0
VZV	13	1	1

LUMC: Loyola University Medical Center; ME: Meningitis/Encephalitis; N.T.: Not Tested

Table 2. Comparison in Enterovirus Detection Between Ordered Tests and The FilmArray ME Panel

Sample ID	Enterovirus		
	Laboratory Test	FilmArray ME	Sequencing Confirmation
pilotLoyola-0007*	+	+	+
pilotLoyola-0015	N.O.	+	+
pilotLoyola-0078	-	-	-
pilotLoyola-0080	-	-	-
pilotLoyola-0098	+	+	+
pilotLoyola-0104	-	+	+
pilotLoyola-0109	+	+	+
pilotLoyola-0110	+	+	+
pilotLoyola-0112	N.O.	+	+

*Co-detection with EBV; N.O.: Not Ordered

Positive results were confirmed with independent PCR followed by sequencing.

The FilmArray ME Panel was concordant with all ordered Enterovirus tests and detected virus in two samples for which no Enterovirus test was ordered.

Figure 4. Enterovirus and EBV Detection in Sample pilotLoyola-0007 with The FilmArray ME Panel

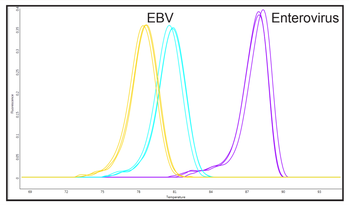


Table 3. Comparison in EBV Detection Between Ordered Tests and The FilmArray ME Panel

Sample ID	EBV		
	Laboratory Test	FilmArray ME	Sequencing Confirmation
pilotLoyola-0007*	N.O.	+	+
pilotLoyola-0010	-	-	-
pilotLoyola-0011	-	-	-
pilotLoyola-0022	-	-	-
pilotLoyola-0039	-	-	+
pilotLoyola-0044	-	-	-
pilotLoyola-0054	-	-	-
pilotLoyola-0093	N.O.	-	+
pilotLoyola-0114	-	-	-
pilotLoyola-0123	-	-	-
pilotLoyola-0132	-	-	-
pilotLoyola-0142	-	-	-
pilotLoyola-0157	N.O.	+	-
pilotLoyola-0163	-	-	-
pilotLoyola-0173	-	-	-

*Co-detection with Enterovirus; N.O.: Not Ordered

Positive results were confirmed with independent PCR followed by sequencing.

The FilmArray ME Panel was concordant with all ordered EBV tests and detected virus in two samples for which no EBV test was ordered.

PCR sequencing detected EBV in two samples that were negative for EBV by FilmArray ME testing.

One FilmArray ME EBV positive detection could not be confirmed with PCR sequencing.

Table 4. Comparison in HHV-6 Detection Between Ordered Tests and The FilmArray ME Panel

Sample ID	HHV-6		
	Laboratory Test	FilmArray ME	Sequencing Confirmation
pilotLoyola-0002	-	-	-
pilotLoyola-0004	-	-	-
pilotLoyola-0010	-	-	-
pilotLoyola-0022	-	-	-
pilotLoyola-0030	N.O.	+	+
pilotLoyola-0032	-	-	-
pilotLoyola-0044	-	-	-
pilotLoyola-0054	N.O.	+	+
pilotLoyola-0081	-	-	-
pilotLoyola-0091	N.O.	+	-
pilotLoyola-0110*	N.O.	-	+
pilotLoyola-0115	N.O.	+	+
pilotLoyola-0121	-	-	-
pilotLoyola-0169	-	-	-

*FilmArray ME Detected Enterovirus; N.O.: Not Ordered

Positive results were confirmed with independent PCR followed by sequencing.

The FilmArray ME Panel was concordant with all ordered HHV-6 tests and detected virus in four samples for which no HHV-6 test was ordered.

One FilmArray ME HHV-6 positive could not be confirmed with PCR sequencing.

Figure 5. HHV-6 Detection in Sample pilotLoyola-0054 by The FilmArray ME Panel

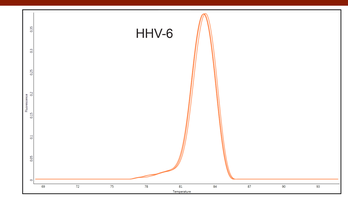


Table 5. Comparison in VZV Detection Between Ordered Tests and The FilmArray ME Panel

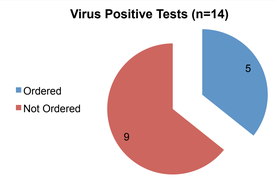
Sample ID	VZV		
	Laboratory Test	FilmArray ME	Sequencing Confirmation
pilotLoyola-0010	-	-	-
pilotLoyola-0011	-	-	-
pilotLoyola-0022	-	-	-
pilotLoyola-0039	-	-	-
pilotLoyola-0044	-	-	-
pilotLoyola-0056	-	-	-
pilotLoyola-0081	-	-	-
pilotLoyola-0097	-	-	-
pilotLoyola-0121	-	-	-
pilotLoyola-0159	-	-	-
pilotLoyola-0161	-	-	-
pilotLoyola-0163	-	-	-
pilotLoyola-0173	+	+	+

*FilmArray ME Detected Enterovirus; N.O.: Not Ordered

Positive results were confirmed with independent PCR followed by sequencing.

The FilmArray ME Panel was concordant with all ordered VZV tests.

Figure 6. Proportion of Virus Positive Test Results Based on Clinical Ordering Practices



67% of CSF virus positive tests were missed because they were not ordered.

CONCLUSIONS

These results suggest that the FilmArray ME Panel has the potential to identify additional pathogens compared to current clinician ordering practices. The significance of these detections in meningitis or encephalitis is unknown. However, providing a comprehensive result in these life-threatening infections may facilitate better patient care through improved antibiotic/antiviral stewardship or intensive care management.

This abstract contains information regarding assays that have not been cleared by the FDA for *in vitro* diagnostic use.

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