Detection of Viral Pathogens in CSF by the FilmArray™ ME Panel

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INTRODUCTION/BACKGROUND

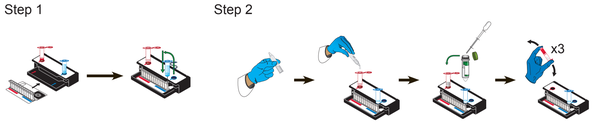
Viral, bacterial, and fungal meningitis present with similar symptoms; however, they require unique treatment strategies. Early, effective treatment is critical for patient survival and recovery. The absence of prompt methods to identify etiological agents of meningitis and encephalitis promotes empirical treatment of suspected bacterial meningitis with antibiotics prior to organism identification. A rapid, fully automated, integrated sample preparation and pathogen detection system, the FilmArray Meningitis/Encephalitis (ME) Panel, is being developed to detect multiple pathogens, including viruses, bacteria, and fungi, from a single CSF sample in about an hour. Limited sample volume and low organism abundance pose challenges to sample preparation in the FilmArray ME Panel, which employs automated fluidic processing. A sample preparation program designed specifically for the ME Panel successfully utilized mechanical lysis and silica-paramagnetic bead nucleic acid purification to prepare RNA and DNA from a small volume of unconcentrated CSF for PCR-based pathogen identification.

MATERIALS AND METHODS

- 8 viral, 6 bacterial, and 2 fungal pathogens known to cause Meningitis or Encephalitis were tested in this study.
- For all bacterial and fungal pathogens tested, fresh cultures were grown and enumerated before being incorporated into the testing.
- Testing of viral targets was performed using enumerated stocks.
- Organisms were divided into 2 pools of 8 organisms per pool for testing in FilmArray ME pouches. Pools were organized in a way to separate similar organisms, as well as to test all organism types (virus, bacteria, fungus) in each pool.
- Each pool was serially diluted and spiked into 5 (20 total) unique CSF samples to determine whether multiple pathogens can be detected within a single sample, as well as to characterize the effects of CSF sample background on detection.
- Concentration by spin columns and precipitation methods was tested on contrived and clinical CSF samples.

Sample Processing and Pouch Loading Instruction

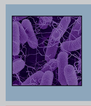
Step 1 Step 2



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

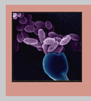
THE FILMARRAY MENINGITIS/ENCEPHALITIS (ME) PANEL

Simultaneous detection of 16 targets:



Bacteria

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



Fungi

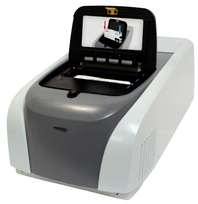
- *Cryptococcus neoformans*
- *Cryptococcus gattii*

Viruses

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

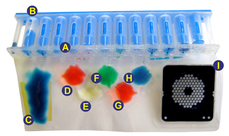
The FilmArray System

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

RESULTS

- All organisms on the ME Panel were detected.
- Detection ranged between 10^2-10^4 for most organisms.
- 100% detection of multiple organisms within one sample. This study confirmed detection of 8 organisms simultaneously.
- Mechanical lysis by bead beating increased performance of the FilmArray ME Panel.
- RNA and DNA targets were effectively isolated from CSF for downstream detection in the FilmArray ME Panel.
- Concentration of CSF was not necessary for detection.
- A minimal CSF volume of 200 mL was sufficient for detection.
- Blood contamination of up to 50% in CSF did not hamper performance.
- CSF and PBST performed similarly in the FilmArray ME Panel.

Table 1. All Organisms Detected at 100% in FilmArray ME

	Organism	CFU/mL or $TCID_{50}$/mL			
		10^1	10^2	10^3	10^4
Viruses	VZV	100%	-	-	-
	HSV1	100%	100%	-	-
	EV	100%	100%	-	-
	CMV (1.2x)	100%	100%	-	-
	HHV6	80%	100%	100%	100%
	HPeV	40%	100%	100%	100%
	EBV (1.7x)	20%	0%	100%	100%
	HSV2*	-	-	0%	80%
Bacteria	*N. meningitidis* (1.9x)	0%	100%	100%	100%
	L. monocytogenes	60%	100%	-	-
	H. influenzae	0%	40%	100%	-
	S. pneumoniae	20%	100%	100%	-
	S. agalactiae	0%	60%	100%	100%
	E. coli	20%	60%	100%	100%
Fungi	*C. gattii*	20%	80%	100%	-
	C. neoformans	0%	20%	80%	100%

*Detected at 10^5 $TCID_{50}$/mL

Figure 1. Multiplexed Detection of Viral Pathogens in Polymicrobial Cerebrospinal Fluid

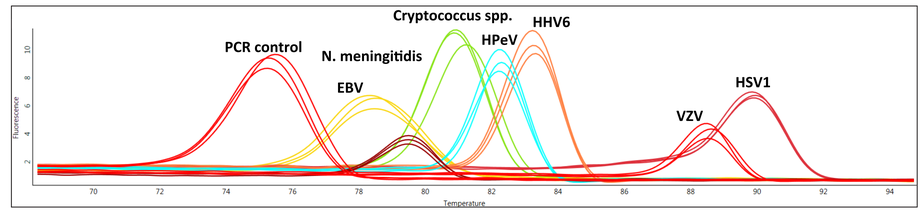
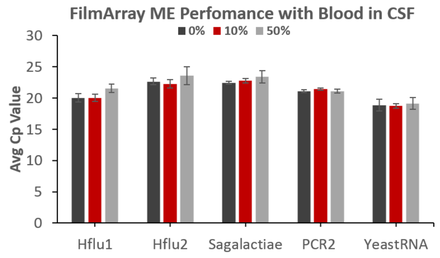


Table 2. Minimal Volume of 200 uL Required for FilmArray ME

ME Assay	CSF Volume (uL)		
	100 uL	200 uL	300 uL
Hflu1	23.7 (8/8)	22.0 (8/8)	22.7 (7/7)
Lmono	22.0 (8/8)	21.3 (8/8)	20.7 (7/7)
Cgattii	18.1 (8/8)	16.8 (8/8)	17.1 (7/7)
HEV1	22.4 (8/8)	20.2 (8/8)	21.1 (7/7)
HEV2	22.3 (8/8)	19.7 (8/8)	21.6 (7/7)
HSV1	20.2 (8/8)	20.0 (8/8)	19.3 (7/7)
YeastRNA	21.2 (8/8)	18.7 (8/8)	20.3 (7/7)
PCR2	20.3 (8/8)	20.3 (8/8)	20.2 (7/7)

C. gattii (10^3 CFU/mL), EV-71 (10^4 $TCID_{50}$/mL), *H. influenzae* (10^3 CFU/mL), HSV1 (10^2 $TCID_{50}$/mL), *L. monocytogenes* (10^3 CFU/mL)

Figure 2. Blood Contamination of CSF Does Not Inhibit Detection



H. influenzae (10^3 CFU/mL), *S. agalactiae* (10^3 CFU/mL)

Figure 3. 60 Second Bead Beating Time Optimal for Lysis of ME Organisms

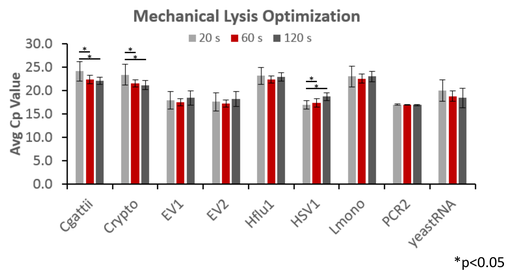


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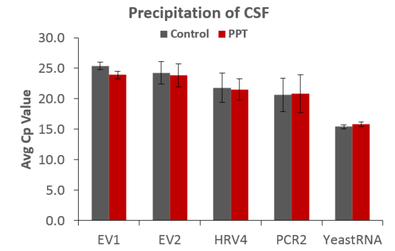
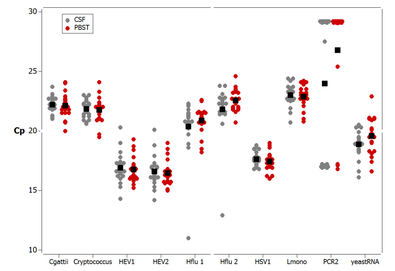
Figure 4. Precipitation of CSF Samples is Not Necessary



EV-71 (10^4 $TCID_{50}$/mL)

Figure 5. CSF and PBST Performed Similarly in FA ME Panel



C. gattii (10^3 CFU/mL), EV-71 (10^4 $TCID_{50}$/mL), *H. influenzae* (10^3 CFU/mL), HSV1 (10^2 $TCID_{50}$/mL), *L. monocytogenes* (10^3 CFU/mL)

CONCLUSIONS

- Development of an automated, integrated sample-to-result purification and detection method of DNA and RNA from a diverse set of pathogens in CSF was achieved in the FilmArray ME Panel.
- A minimal volume (200 mL) of unconcentrated CSF combined with mechanical lysis proved to be an efficient sample preparation method, providing sensitive detection of the most common etiological agents of meningitis and encephalitis.
- Rapid detection (~60 mins) of pathogens in CSF by the FilmArray ME Panel will facilitate prompt, appropriate patient treatment and mitigate empirical antibiotic use, both which benefit patient outcomes and antibiotic stewardship.
- The FilmArray ME Panel has not been CE-marked or US FDA-cleared for In Vitro Diagnostic Use.

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