Evaluation of a Multiplex PCR Panel for the Rapid Detection of Viral, Bacterial and Fungal Pathogens in Cerebrospinal Fluid

#1068

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INTRODUCTION/BACKGROUND

Central nervous system (CNS) infections are responsible for causing inflammatory conditions of the brain and/or meningeal tissues surrounding the brain. Approximately 15% of cases are fatal and many other cases result in life-long disabilities such as loss of limbs, visual and hearing deficits, seizures, and altered learning and memory. Early and effective treatment of meningitis and/or encephalitis is critical to reducing morbidity and mortality however, determining the etiological agent(s) of the infection remains a significant challenge due to current diagnostic methodologies that are difficult and time consuming to perform.

The FilmArray® Meningitis/Encephalitis (ME) Panel (BioFire Diagnostics, LLC) is a fully automated and user-friendly pathogen detection platform for the simultaneous detection of 15 potential ME pathogens (bacteria, viruses and yeast) from 200 µL of cerebrospinal fluid (CSF) specimen. The FilmArray integrates nucleic acid purification, reverse transcription, and nested multiplex PCR amplification with high resolution DNA melt analysis for analyte detection into one closed system. Testing requires less than 2 minutes of hands-on time and approximately one hour to obtain results in an automated report format. The FilmArray ME Panel detects the following organisms:

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*

Viruses

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

Yeast

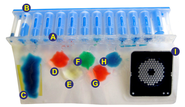
- *Cryptococcus neoformans/gattii*

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

A series of non-clinical (analytical) studies were conducted to establish the performance characteristics of an IUO (Investigational Use Only) version of the FilmArray ME Panel. Studies determined the limit of detection (LoD) for each analyte, reactivity and analytical specificity of the assays, reproducibility of results, as well as the effect of potential interfering substances on system performance.

LIMIT OF DETECTION

Limit of Detection (LoD), is the lowest concentration at which an analyte is consistently detected in ≥95% of samples and was established for each FilmArray ME Panel analyte (Table 1).

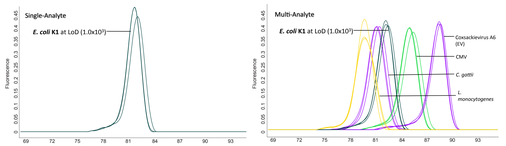
LoD was determined to be 1.0x10^3 CFU/mL or less for bacterial and fungal analytes and LoD for viral analytes was between ~1.0x10^3 - 1.0x10^4 nucleic acid copies/mL or 5 - 500 TCID$_{50}$/mL.

Table 1. LoD for FilmArray ME Panel Analytes

ME Panel Test Results	Species/Isolate Tested	LoD Concentration
BACTERIA		
E. coli K1	ATCC 700973	1.0x10^3 CFU/mL
H. influenzae	ATCC 10211	1.0x10^3 CFU/mL
L. monocytogenes	ATCC 13932	1.0x10^3 CFU/mL
N. meningitidis	ATCC 43744	1.0x10^3 CFU/mL
S. agalactiae	ATCC 13813	1.0x10^3 CFU/mL
S. pneumoniae	ATCC 33400	100 cells/mL
VIRUSES		
CMV	Zeptometrix 0810003CF	100 TCID$_{50}$/mL (4.3 x10^3 copies/mL)
EV (Species A-D)	**Coxsackievirus A6**, Species A ATCC VR-1801 **Coxsackievirus A9**, Species B Zeptometrix 0810017CF **Coxsackievirus A17**, Species C ATCC VR-1023 **Enterovirus 70**, Species D ATCC VR-836	5 - 50 TCID$_{50}$/mL
EBV	Zeptometrix 0810008CF	1.0x10^4 copies/mL
HSV-1	Zeptometrix 0810005CF	250 TCID$_{50}$/mL (1.5x10^5 copies/mL)
HSV-2	Zeptometrix 0810006CF	50 TCID$_{50}$/mL (1.3x103^5 copies/mL)
HHV-6	**HHV-6A**, NCPV 0003121v **HHV-6B**, NCPV 0006111v	1.0x10^4 copies/mL
HPeV	Zeptometrix 0810147CF	500 TCID$_{50}$/mL
VZV	Zeptometrix 0810171CF	0.1 TCID$_{50}$/mL (1.7x10^5 copies/mL)
YEAST		
Cryptococcus neoformans/gattii	***C. neoformans***, ATCC 208821 ***C. gattii***, ATCC MYA-4877	100 CFU/mL

A comparison of analyte detection in single-analyte and multi-analyte contrived samples was also performed (Figure 1). Detection of individual analytes at concentrations near LoD was equivalent in samples containing one or multiple analytes.

Figure 1. A Comparison of Analyte Detection (by Melting Curve Analysis[a]) for Single-Analyte and Multi-Analyte Samples



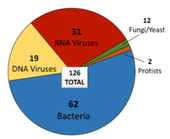
[a]Melt curves are provided in triplicate per assay.

EXCLUSIVITY AND INCLUSIVITY

Testing was performed to demonstrate that the ME Panel can detect all strains of the analytes targeted by the ME Panel (inclusivity) while excluding cross-reactivity with near-neighbors and other clinically relevant non-target organisms (exclusivity).

Exclusivity of the ME Panel assays was evaluated by testing high concentrations of 126 on-panel and off-panel organisms (Figure 2). On-panel organisms were tested to demonstrate a lack of inter-assay or intra-panel cross-reactivity. Off-panel organisms were selected for testing based on one or more of the following:

Figure 2. Organisms Tested for Exclusivity



- Relatedness (near-neighbors) to species detected by the ME Panel
- Clinical relevance
- Likelihood of being present in CSF specimens
- Genetic similarity to ME Panel assay primers

Three FilmArray ME Panel assays were found to cross-react with closely related species that are rarely or never isolated from human CSF (Table 3), indicating only a very minor risk of inaccurate FilmArray ME Panel results due to cross-reactivity. No other cross reactivity was observed or predicted by in silico analysis for the FilmArray ME Panel assays.

Table 2. Cross-Reactivity between ME Panel Assays and Off-Panel Organisms

FilmArray ME Panel Test Result	Cross-Reactive Organism
Haemophilus influenzae	*Haemophilus haemolyticus*[a]
Enterovirus	Rhinovirus (multiple serotypes)
Cryptococcus neoformans/gattii	*Cryptococcus amylolentus*[b]

[a]Cross-reactivity was observed only at concentrations >10^5 CFU/mL.
[b]Not isolated from humans (normal habitat is insect frass).

Inclusivity of the FilmArray ME Panel assays was evaluated by testing 98 unique isolates of clinically relevant species, serotypes and genotypes at concentrations near LoD, as well as *in silico* analyses of available organism sequences (Table 3).

Table 3. Inclusivity for FilmArray ME Panel Assays

ME Panel Test Result	# of Isolates Tested	Concentration Detected	Description of ME Panel Reactivity (includes predictions from in silico analysis[a])
BACTERIA			
E. coli K1	5	≤ 3.0x10^3 CFU/mL	Detects *E. coli* strains of the K1 serotype only
H. influenzae	9	≤ 3.0x10^3 CFU/mL	Detects non-typeable and typeable (types a-f) strains of *H. influenzae*
L. monocytogenes	6	≤ 3.0x10^3 CFU/mL	Detects all types of *L. monocytogenes*
N. meningitidis	7	≤ 3.0x10^3 CFU/mL	Detects all serotypes of encapsulated *N. meningitis*. Unencapsulated strains will not be detected
S. agalactiae	5	≤ 3.0x10^3 CFU/mL	Detects all serotypes of *S. agalactiae* (Group B *Streptococcus*)
S. pneumoniae	6	≤ 300 cells/mL	Detects all serotypes of *S. pneumoniae*
VIRUSES			
CMV	5	≤ 300 TCID$_{50}$/mL (≤ 1.0×10^4 copies/mL)	Detects all Cytomegalovirus (CMV)
EV (Species A-D)	18	≤ 50 TCID$_{50}$/mL	Detects all species (A-D) and serotypes (>100) of human EV
EBV	2	≤ 3.0×10^4 copies/mL	Detects all EBV
HSV-1	5	≤ 750 TCID$_{50}$/mL (≤ 4.5×10^5 copies/mL)	Detects all HSV-1
HSV-2	5	≤ 150 TCID$_{50}$/mL (≤ 4.0×10^3 copies/mL)	Detects all HSV-2
HHV-6	4	≤ 3.0×10^4 copies/mL	Detects A and B variants of HHV-6
HPeV	6	500 – 5.0x10^3 TCID$_{50}$/mL	Detects serotypes 1-8 of HPeV[b]
VZV	5	≤ 0.3 TCID$_{50}$/mL (≤ 5.0×10^3 copies/mL)	Detects all VZV
YEAST			
Cryptococcus neoformans/gattii	10 (5 per species)	≤ 300 CFU/mL	Detects *C. neoformans* and *C. gattii*, but will not distinguish between species

[a]Assay primers were evaluated for reactivity against sequences available from public databases for organisms detected by the panel.
[b]Serotypes 1-6 were tested and detected, serotypes 7 and 8 predicted to be detected by in silico analysis.

REPRODUCIBILITY

Reproducibility of the FilmArray ME Panel was evaluated by testing contrived samples containing various combinations of ME Panel analytes at one of three concentrations (moderate positive, low positive and negative (no analyte)). Samples were tested by different operators (6), on three different FilmArray 2.0 systems (19 total instruments), with different kit lots (3), on five different days (Table 5). The study included 360 valid test runs with 5,400 total possible test results. Reproducibility of the panel was calculated for each of the three systems (A, B and C) and overall (All Systems) as the percent agreement between the expected test results and the results obtained (Table 6).

The FilmArray ME Panel test results were reproducible within and between systems and the other variables evaluated and overall agreement with the expected results was high (99.7% overall (5,386/5,400) with a 95% confidence interval of 99.6-99.9%).

Table 5. Reproducibility of the FilmArray ME Panel Test Results

Analyte	Concentration Tested	Expected Result	Agreement with Expected Result			
			Sys A	Sys B	Sys C	All Systems (95% CI)
BACTERIA						
E. coli K1	**Moderate Positive 3× LoD 3.0×10^3 CFU/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1.0×10^3 CFU/mL	Detected	29/30 96.7%	29/30 96.7%	29/30 96.7%	**87/90 96.7%** (90.6%-99.3%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
H. influenzae	**Moderate Positive 3× LoD 3.0×10^3 CFU/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1.0×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
L. monocytogenes	**Moderate Positive 3× LoD 3.0×10^3 CFU/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 1.0×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
N. meningitidis	**None (no analyte)**	Not Detected	120/120 100%	120/120 100%	120/120 100%	**360/360 100%** (99.0%-100%)
S. agalactiae	**Moderate Positive 3× LoD 3.0×10^3 CFU/mL**	Detected	29/30 96.7%	30/30 100%	29/30 96.7%	**88/90 97.8%** (92.2%-99.7%)
	Low Positive 1× LoD 1.0×10^3 CFU/mL	Detected	29/30 96.7%	29/30 96.7%	30/30 100%	**88/90 97.8%** (92.2%-99.7%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
S. pneumoniae	**None (no analyte)**	Not Detected	120/120 100%	120/120 100%	120/120 100%	**360/360 100%** (99.0%-100%)
VIRUSES						
CMV	**None (no analyte)**	Not Detected	120/120 100%	120/120 100%	120/120 100%	**360/360 100%** (99.0%-100%)
EV (Coxsackievirus A9)	**Moderate Positive 3× LoD 15 TCID$_{50}$/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 5 TCID$_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
EBV	**Moderate Positive 3× LoD 3.0×10^4 copies/mL**	Detected	30/30 100%	30/30 100%	29/30 96.7%	**89/90 98.9%** (94.0%-100%)
	Low Positive 1× LoD 1.0×10^4 copies/mL	Detected	29/30 96.7%	30/30 100%	29/30 96.7%	**88/90 97.8%** (92.2%-99.7%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
HSV-1	**None (no analyte)**	Not Detected	120/120 100%	120/120 100%	120/120 100%	**360/360 100%** (99.0%-100%)
HSV-2	**Moderate Positive 3× LoD 150 TCID$_{50}$/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 50 TCID$_{50}$/mL	Detected	30/30 100%	30/30 100%	29/30 96.7%	**89/90 98.9%** (94.0%-100%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
HHV-6	**None (no analyte)**	Not Detected	120/120 100%	120/120 100%	120/120 100%	**360/360 100%** (99.0%-100%)
HPeV	**Moderate Positive 3× LoD 1.5×10^3 TCID$_{50}$/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 500 TCID$_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
VZV	**Moderate Positive 3× LoD 0.30 TCID$_{50}$/mL**	Detected	30/30 100%	30/30 100%	29/30 96.7%	**89/90 98.9%** (94.0%-100%)
	Low Positive 1× LoD 0.10 TCID$_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
YEAST						
Cryptococcus neoformans/gattii	**Moderate Positive 3× LoD 300 CFU/mL**	Detected	30/30 100%	30/30 100%	30/30 100%	**90/90 100%** (96.0%-100%)
	Low Positive 1× LoD 100 CFU/mL	Detected	30/30 100%	30/30 100%	28/30 93.3%	**88/90 97.8%** (92.2%-99.7%)
	None (no analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	**180/180 100%** (98.0%-100%)
Overall Agreement with the Expected Test Result for All Analytes and All Test Levels (95% Confidence Interval)			**5,386/5,400 99.7% (99.6% – 99.9%)**			

INTERFERING SUBSTANCES

Substances that could be inherent to CSF or introduced during collection of CSF specimens and have the potential to interfere with the performance of the FilmArray ME Panel were added to contrived CSF samples containing representative panel organisms at 3× LoD. Each substance was tested at or above clinically relevant concentrations and was evaluated for its potential to cause control failures or inaccurate test results.

The potential for interference was observed only from protein at concentrations higher than expected in CSF and from damage to the sample prior to testing caused by bleach (Table 4).

Table 6. Results of Potentially Interfering Substances Testing

Substance	Reference Concentration		Tested Concentration	Result
	Normal	Meningitis/Encephalitis		
Glucose	40-70 mg/dL[1]	≤ 70 mg/dL[2]	990 mg/dL	No Interference
Lactate	10-20 mg/dL[1]	> 30 mg/dL[3]	220 mg/dL	No Interference
Protein (Albumin)	45 mg/dL[2]	50-500 mg/dL[2]	5.0x10^3 mg/dL	Partial Interference[a]
			4.0x10^3 mg/dL	Partial Interference[a]
			1.5x10^3 mg/dL	No Interference
			500 mg/dL	No Interference
			100 mg/dL	No Interference
Immunoglobulin (IgG)	0-8.0 mg/dL[4]	> 8.0 mg/dL	1.0x10^3 mg/dL	No Interference
White Blood Cells (WBC)	0-20 cells/µL[5]	5-5,000 cells/µL[1]	1.0x10^4 cells/µL	No Interference
Human Genomic DNA	≤ 0.068 ng/µL	≤ 17 ng/µL	20 ng/µL	No Interference
Human Whole Blood	None[b]		10% (v/v)	No Interference
Hemoglobin	None[a]		200mg/dL	No Interference
			(2 mg/mL)	No Interference
Trans-Isolate (T-I) Medium	None		50% (v/v)	No Interference
Viral Transport Medium (VTM)	None		50% (v/v)	No Interference
Ethanol	None		7% (v/v)	No Interference
Bleach	None		1% (v/v)	Interference[c]
	None		0.1% (v/v)	No Interference
	None		0.01% (v/v)	No Interference

[a]Partial interference by albumin was observed (Not Detected results for one or more analytes in a sample) only at concentrations substantially greater than the highest total protein levels expected in a CSF specimen.

[b]Blood (and hemoglobin) are not normally in CSF but may be present due to a bloody tap (lumbar puncture) or subarachnoid hemorrhage.

[c]No organisms were detected despite normal pouch function (control assays passed). Further investigation indicated bleach-associated damage to nucleic acids (oxidation or other damage) in the sample prior to testing.

CONCLUSIONS

Non-clinical studies have demonstrated that the FilmArray ME Panel is a sensitive, robust and reproducible system for molecular detection of ME pathogens in CSF specimens.

- The ME Panel has the capability to accurately identify bacterial, viral and fungal ME pathogens from one CSF specimen (including polymicrobial CSF specimens) in one rapid test (approximately one hour).
- The ME Panel assays are inclusive for clinically important variants of ME pathogens and exclusive of most other potential pathogens or CSF contaminants.
- The simple set-up and integrated sample lysis, nucleic acid purification, nested multiplex RT-PCR and melting analysis leads to highly reproducible test results in the hands of multiple users, systems, and other variables.
- The ME Panel specimen to answer process is tolerant of blood and other potential PCR inhibitors that may be in CSF specimens, though care should be taken to avoid damage caused by exposure of specimens to bleach.

The FilmArray ME Panel has the potential to improve meningitis/encephalitis diagnostic testing by providing faster and more comprehensive results than current diagnostic laboratory practices.

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

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