Multi-Center Clinical Evaluation of a Multiplex Meningitis/Encephalitis PCR Panel for Simultaneous Detection of Bacteria, Yeast, and Viruses in Cerebrospinal Fluid Specimens

#1074

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INTRODUCTION/BACKGROUND

The FilmArray® Meningitis/Encephalitis (ME) Panel (Investigational Use Only; BioFire Diagnostics) 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 into one closed system. Testing requires less than 2 minutes of hands-on time and approximately one hour to results (including an automated report).

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

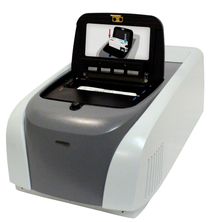
- Cytomegalovirus
- Enterovirus
- Epstein-Barr virus
- Herpes simplex virus 1
- Herpes simplex virus 2
- Human herpesvirus 6
- Human parechovirus
- Varicella zoster virus

Yeast

- *Cryptococcus neoformans/gattii*

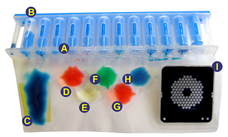
The FilmArray System

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®.



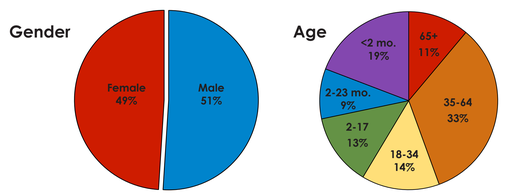
A. Fitment with freeze-dried reagents
B. Plungers- deliver reagents to blisters
C. Sample lysis and bead collection
D. Wash station
E. Magnetic bead collection blister
F. Elution Station
G. Multiplex Outer PCR blister
H. Dilution blister
I. Inner Nested PCR array

CLINICAL PERFORMANCE EVALUATION

The clinical performance of the FilmArray ME Panel was established during a prospective multi-center study conducted at 11 U.S. study sites between February and September, 2014 (1560 CSF specimens).

Figure 1. Clinical Trial Sites

Figure 2. Demographic Analysis



For each specimen, the FilmArray ME Panel results were compared to standard laboratory methods for identification of bacterial analytes (CSF culture performed by the clinical study sites) as well as polymerase chain reaction (PCR)/sequencing-based comparator methods for identification of viruses and yeast.

Table 1. Results Comparison Method: FilmArray ME Panel and Comparator

	Assay Result	
Comparison Result	**ME Panel**	**Comparator**
True Positive (TP)	+	+
False Positive (FP)	+	-
True Negative (TN)	-	-
False Negative (FN)	-	+

The clinical sensitivity or positive percent agreement (PPA) between FilmArray and the comparison method was calculated as 100% x (TP / (TP + FN)). The clinical specificity or negative percent agreement (NPA) was calculated as 100% x (TN / (TN + FP)). The binomial two-sided 95% confidence interval (95% CI) was calculated.

Table 2. Prospective Clinical Performance[a]

Analyte	Sensitivity			Specificity		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Bacteria						
E. coli K1	2/2	100	34.2-100	1557/1558	99.9	99.6-100
H. influenzae	1/1	100	-	1558/1559[b]	99.9	99.6-100
L. monocytogenes	0/0	-	-	1560/1560	100	99.8-100
N. meningitidis	0/0	-	-	1560/1560	100	99.8-100
S. agalactiae	0/1[c]	0.0	-	1558/1559	99.9	99.6-100
S. pneumoniae	4/4	100	51.0-100	1544/1556[d]	99.2	98.7-99.6
Analyte	**Positive Percent Agreement**			**Negative Percent Agreement**		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Viruses						
CMV	3/3	100	43.9-100	1554/1557[e]	99.8	99.4-99.9
EV	44/46[f]	95.7	85.5-98.8	1507/1514[f]	99.5	99.0-99.8
EBV	18/22[g]	81.8	61.5-92.7	1515/1538[g]	98.5	97.8-99.0
HSV-1	2/2	100	34.2-100	1556/1558	99.9	99.5-100
HSV-2	10/10	100	72.2-100	1548/1550[h]	99.9	99.5-100
HHV-6	18/21[i]	85.7	65.4-95.0	1532/1536[i]	99.7	99.3-99.9
HPeV	9/9	100	70.1-100	1548/1551[j]	99.8	99.4-99.9
VZV	4/4	100	51.0-100	1553/1556[k]	99.8	99.4-99.9
Yeast						
C. neoformans/gattii	1/1	100	-	1555/1559[l]	99.7	99.3-99.9

[a] The performance measures of sensitivity and specificity only refer to bacterial analytes for which the gold-standard of CSF bacterial culture was used as the reference method. Performance measures of PPA and NPA refer to all other analytes, for which PCR/sequencing assays were used as comparator methods.
[b] *H. influenzae* was detected in the single FP specimen using an independent molecular method and was also observed via Gram stain; the subject from whom this specimen was collected received a physician diagnosis of gram-negative bacterial meningitis.
[c] The laboratory reported that *S. agalactiae* was present at a very low level (two colonies) for the FN specimen.
[d] *S. pneumoniae* was detected in 5/12 FP specimens using an independent molecular method.
[e] CMV was detected in 1/3 FP specimens using an independent molecular method.
[f] EV was detected in 2/2 FN specimens using an independent molecular method; one specimen was positive upon FilmArray ME retest. EV was detected in 5/7 FP specimens using an independent molecular method.
[g] EBV was detected in 2/4 FN and 4/23 FP specimens using an independent molecular method.
[h] HSV-2 was detected in 1/2 FP specimens using an independent molecular method; the subject from whom this specimen was collected received a physician diagnosis of HSV meningitis.
[i] HHV-6 was detected in 2/3 FN and 1/4 FP specimens using an independent molecular method.
[j] HPeV was detected in 1/3 FP specimens using an independent molecular method; the subject from whom this specimen was collected received a physician diagnosis of HPeV meningitis. Both of the subjects from whom the remaining two specimens were collected received a diagnosis of HPeV infection following detection of HPeV in the blood.
[k] VZV was detected in 1/3 FP specimens using an independent molecular method; the subject from whom this specimen was collected received a physician diagnosis of herpes zoster. Of the remaining two specimens with FP results, one was collected from a subject who was diagnosed with herpes zoster oticus.
[l] *C. neoformans/gattii* was detected in 2/4 FP specimens using a commercial antigen test; both subjects from whom these specimens were collected received a physician diagnosis of cryptococcal meningitis.

All but three of the organism results demonstrated a sensitivity/PPA of 95.7% or higher. The three exceptions were *Streptococcus agalactiae* (0%), EBV (81.8%), and HHV-6 (85.7%). Due to low prevalence, only one of 13 of the other analytes (EV) demonstrated a lower bound of the two-sided 95%CI of 80.0% or higher. All organism results demonstrated specificity/NPA of 98.5% or greater, with lower bounds of the 95%CI of 97.8% or greater.

With the exception of Enterovirus, all of the analytes detected by the ME Panel were of low prevalence, therefore, 235 preselected clinical archived specimens were evaluated.

The FilmArray ME Panel reported a total of 14 specimens with discernible multiple organism detections (0.9% of all specimens, 14/1560; and 8.4% of positive specimens, 14/167. The majority of multiple detections (13/14; 92.9%) contained two organisms, while 7.1% (1/14) contained three organisms.

The three organisms that were most prevalent in co-detections were EBV, *S. pneumoniae*, and HSV-2. The most prevalent multiple detection was EBV with HSV-2 (0.2% of all specimens; 3/1560). All but one co-detection was associated with a herpesvirus, and half (seven) were associated with EBV specifically.

Figure 3. Prospective Clinical FilmArray Detections

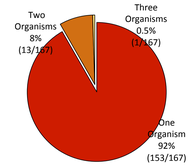


Table 3. FilmArray ME Panel Multiple Detection Combinations

Multiple Detection Combination	Number of Specimens
EBV + HSV-2	3
EBV + *S. pneumoniae*	2
EBV + *S. pneumoniae* + VZV	1
CMV + EBV	1
CMV + *S. pneumoniae*	1
EV + EBV	1
EV + HPeV	1
EBV + HSV-1	1
EBV + VZV	1
HSV-1 + HHV-6	1
HSV-2 + *S. agalactiae*	1

Table 4. Archived Specimen Performance

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95%CI	TN/(TN + FP)	%	95%CI
Bacteria						
E. coli K1	2/2	100	34.2-100	35/35	100	90.1-100
H. influenzae	3/3	100	43.9-100	39/39	100	91-100
L. monocytogenes	1/1	100	-	41/41	100	91.4-100
N. meningitidis	7/7	100	64.6-100	34/34	100	89.8-100
S. agalactiae	2/2	100	34.2-100	40/40	100	91.2-100
S. pneumoniae	17/17	100	81.6-100	21/21	100	84.5-100
Viruses						
CMV	7/8	87.5	52.9-97.8	181/181	100	97.9-100
EBV	12/14	85.7	60.1-96.0	137/145	94.5	89.5-97.2
HSV-1	16/16	100	80.6-100	156/157	99.4	96.5-99.9
HSV-2	33/34	97.1	85.1-99.5	136/136	100	97.3-100
HHV-6	12/16[a]	75.0	50.5-89.8	168/168	100	97.8-100
HPeV	2/3	66.7	20.8-93.9	187/187	100	98.0-100
VZV	22/22	100	85.1-100	162/164	98.8	95.7-99.7
Yeast						
C. neoformans/gattii	19/19[b]	100	83.2-100	171/171	100	97.8-100

[a] Two specimens were identified as positive for HHV-6A while 14 were found positive for HHV-6B. Of the four FilmArray FN specimens, one was identified as positive for HHV-6A and the remaining three FN specimens were identified as positive for HHV-6B. The resulting PPA was 50% (1/2); 95% CI 9.5 – 90.5% and 79% (11/14); 95% CI 52.4 – 925.4% for HHV-6A and HHV-6B, respectively.
[b] One specimen was positive for *C. gattii* and 18 were found positive for *C. neoformans*.

CONCLUSIONS

Overall, the FilmArray ME Panel is a highly sensitive, specific and robust test for infectious bacterial, viral and yeast agents of meningitis and encephalitis illness.

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

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