EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
FilmArray® Meningitis/Encephalitis (ME) Panel

DECISION SUMMARY

A. DEN Number:

DEN150013

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the FilmArray Meningitis/Encephalitis (ME) Panel

C. Measurands:

The assay detects and identifies nucleic acids of the following organisms: *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis* (encapsulated), *Streptococcus agalactiae*, *Streptococcus pneumoniae*, Cytomegalovirus, Enterovirus, Herpes simplex virus 1, Herpes simplex virus 2, Human herpesvirus 6, Human parechovirus, Varicella zoster virus, *Cryptococcus neoformans/gattii*

D. Type of Test:

The FilmArray Meningitis/Encephalitis (ME) Panel ("FilmArray ME Panel"), performed with FilmArray and FilmArray 2.0 systems, is a nucleic acid-based test for the detection of the above listed bacteria, viruses, and yeast from cerebrospinal fluid (CSF) specimens obtained from patients with signs and symptoms of meningitis or encephalitis.

E. Applicant:

BioFire Diagnostics, LLC

F. Proprietary and Established Names:

FilmArray Meningitis/Encephalitis (ME) Panel

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3970, Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid

2. Classification:

   Class II (Special Controls)

3. Product code(s):

   PLO, OOI, NSU

4. Panel:

   83- Microbiology

H. Intended Use:

1. Intended use(s):

   The FilmArray Meningitis/Encephalitis (ME) Panel is a qualitative multiplexed nucleic acid-based *in vitro* diagnostic test intended for use with FilmArray and FilmArray 2.0 systems. The FilmArray ME Panel is capable of simultaneous detection and identification of multiple bacterial, viral, and yeast nucleic acids directly from cerebrospinal fluid (CSF) specimens obtained via lumbar puncture from individuals with signs and/or symptoms of meningitis and/or encephalitis. The following organisms are identified using the FilmArray ME Panel:

   Bacteria:

   Escherichia coli K1
   Haemophilus influenzae
   Listeria monocytogenes
   Neisseria meningitidis (encapsulated)
   Streptococcus agalactiae
   Streptococcus pneumoniae

   Viruses:
   Cytomegalovirus
   Enterovirus
   Herpes simplex virus 1
   Herpes simplex virus 2
   Human herpesvirus 6
   Human parechovirus
   Varicella zoster virus

   Yeast:
   Cryptococcus neoformans/gattii

   The FilmArray ME Panel is indicated as an aid in the diagnosis of specific agents of meningitis and/or encephalitis and results are meant to be used in conjunction with other clinical, epidemiological, and laboratory data.

   Results from the FilmArray ME Panel are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out co-infection with organisms not included in the FilmArray ME Panel. The agent

detected may not be the definite cause of the disease. Negative results do not preclude central nervous system (CNS) infection. Not all agents of CNS infection are detected by this test and sensitivity in clinical use may differ from that described in the package insert.

The FilmArray ME Panel is not intended for testing of specimens collected from indwelling CNS medical devices.

The FilmArray ME Panel is intended to be used in conjunction with standard of care culture for organism recovery, serotyping, and antimicrobial susceptibility testing.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   The FilmArray ME Panel is performed on the FilmArray and FilmArray 2.0 systems.

I. Device Description:

The FilmArray ME Panel is a multiplex nucleic acid-based test designed to be used with FilmArray or FilmArray 2.0 system ("FilmArray systems" or "FilmArray instruments"). The FilmArray ME panel includes a FilmArray ME Panel pouch (pouch) which contains freeze-dried reagents to perform nucleic acid purification and nested, multiplex polymerase chain reaction (PCR) with DNA melt analysis. The FilmArray ME Panel simultaneously conducts 14 tests for the identification of potential CNS pathogens from CSF specimens obtained via lumbar puncture. Results from the FilmArray ME Panel are available within about one hour.

A test is initiated by loading Hydration Solution into one port of the pouch and a CSF sample mixed with the provided Sample Buffer ampoules into the other port of the pouch and placing it in the FilmArray Instrument. The pouch contains all of the reagents required for specimen testing and analysis in a freeze-dried format; the addition of Hydration Solution and the Sample Buffer rehydrates the reagents. After the pouch is prepared, the FilmArray Software on the FilmArray systems guides the user though the steps of placing the pouch into the instrument, scanning the pouch barcode, entering the sample identification, and initiating the run on the FilmArray systems.

The FilmArray instruments contain a coordinated system of inflatable bladders and seal points, which act on the pouch to control the movement of liquid between the pouch blisters. When a bladder is inflated over a reagent blister, it forces liquid from the blister into connecting channels. Alternatively, when a seal is placed over a connecting channel it acts as a valve to open or close a channel. b(4)

b(4)

b(4)

Nucleic acid extraction occurs within the pouch using mechanical and chemical lysis followed by purification using standard magnetic bead technology. After extracting and purifying nucleic acids from the unprocessed sample, a nested multiplex PCR is executed in two stages. b(4) . The solution is then distributed to each well of the array. Array wells contain sets of primers designed specifically to amplify sequences internal to the PCR products generated during the first stage PCR reaction. The 2nd stage PCR, or nested PCR, is performed in each well of the array. At the conclusion of the 2nd stage PCR, the array is interrogated by melt curve analysis for the detection of signature amplicons denoting the presence of specific targets. A digital camera placed in front of the array captures fluorescent images of the PCR2 reactions and software interprets the data.

The FilmArray software automatically interprets the results of each DNA melt curve analysis and combines the data with the results of the internal pouch controls to provide a test result for each organism on the panel.

Materials provided in each FilmArray ME Panel kit:

- Individually packaged FilmArray ME Panel pouches
- Single-use (1.0 mL) Sample Buffer ampoules
- Single-use pre-filled (1.5 mL) Hydration Injection Vials
- Single-use Sample Injection Vials
- Individually packaged Transfer Pipettes

Materials required but not provided: FilmArray system including:

- FilmArray or FilmArray 2.0 instrument and software
- FilmArray Pouch Loading Station

J. Standard/Guidance Document Referenced (if applicable):

- CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance, 2008
- CLSI EP07-A2, Interference Testing in Clinical Chemistry, 2005
- CLSI MM03-A2, Molecular Diagnostic Methods for Infectious Diseases, 2006
- EN ISO 14971:2012, 'Medical devices – Application of risk management to medical devices'
- Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff – Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable, April 25, 2005
- Guidance for Industry and Food and Drug Administration Staff – Assay Migration Studies for In Vitro Diagnostic Devices, April 25, 2013
- Guidance for Industry and Food and Drug Administration Staff – Highly Multiplexed

Microbiological/Medical Countermeasure *In Vitro* Nucleic Acid Based Diagnostic Devices, August 27, 2014
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, FDA Guidance Document, March 13, 2007
- Class II Special Controls Guidance Document: Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA, January 2, 2009

K. Test Principle:

The FilmArray ME Panel pouch (pouch) is a closed system disposable that houses all the chemistry required to isolate, amplify and detect nucleic acid from multiple meningitis and encephalitis pathogens within a single CSF specimen obtained from a lumbar puncture. The rigid plastic component (fitment) of the pouch contains reagents in freeze-dried form. The flexible plastic portion of the pouch is divided into discrete segments (blisters) where the required chemical processes are carried out. The user of the FilmArray ME Panel loads the sample into the pouch, places the pouch into the FilmArray systems, and starts the run. All other operations are automated. Operations and processes that occur during a FilmArray run include the following:

- **Nucleic Acid Purification** - b(4) The sample is lysed by agitation (bead beating) and the liberated nucleic acid is captured, washed and eluted using magnetic bead technology. These steps require approximately ten minutes and the bead-beater apparatus can be heard as a high-pitched whine during the first minute of operation.

- **Reverse Transcription and 1st Stage Multiplex PCR** - Some pathogens identified by the pouch are RNA viruses, and a reverse transcription (RT) step is performed to convert the viral RNA into cDNA prior to amplification. b(4) for multiplex PCR. The effect of 1st stage PCR is to enrich for the target nucleic acids present in the sample.

- **2nd Stage PCR** - The products of 1st stage PCR are diluted and mixed with fresh PCR reagents containing an intercalating fluorescent DNA dye b(4) Plus, BioFire Defense, LLC). This solution is distributed over the 2nd stage PCR array. The individual wells of the array contain primers for different assays (each present in triplicate) that target specific nucleic acid sequences from each of the pathogens detected, as well as control template material. These primers are 'nested' or internal to the specific products of the 1st stage multiplex reaction, which enhances both the sensitivity and specificity of the reactions.

- **DNA Melting Analysis** – After 2nd stage PCR, the temperature is slowly increased and fluorescence in each well of the array is monitored and analyzed to generate a melt curve. The temperature at which a specific PCR product melts (melting temperature or Tm) is consistent and predictable and the FilmArray software automatically evaluates the data from replicate wells for each assay to report results.

The FilmArray software controls the operation of the instrument, collects and analyzes data and automatically generates a test report at the end of the run.

L. Performance Characteristics:

1. Analytical performance:

a. Reproducibility

Reproducibility studies were performed with the FilmArray ME Panel on both the FilmArray and FilmArray 2.0 systems. Testing for the FilmArray system was performed using multiple instruments at three different testing sites, Biofire Diagnostics and two external laboratories. Testing for the FilmArray 2.0 system was performed internally at BioFire Diagnostics using multiple instruments at three different locations within BioFire Diagnostics.

Assay reproducibility was evaluated for both the FilmArray and FilmArray 2.0 system using a panel of contrived CSF samples prepared in artificial CSF matrix (aCSF) and spiked with combinations of nine different ME Panel analytes, including at least one representative gram-negative bacterium, gram-positive bacterium, yeast, DNA virus and RNA virus. Each spiked analyte was evaluated at three different concentrations: Negative (no analyte), Low Positive (1× the limit of detection (LoD)) and Moderate Positive (3× LoD). Testing on both FilmArray systems incorporated a range of potential testing variables including different operators, three different pouch lots, and different FilmArray Instruments. Samples were tested on five different days with a total of 90 replicates tested per panel member.

For the FilmArray system, 366 runs were initiated and 360 runs were completed (98.4% initially valid results). Of the six initially invalid runs, one invalid run was due to a control failure and five invalid tests were due to instrument or software errors. Retesting of initially invalid specimens gave valid results for all six specimens. There were seven unexpected false positive results observed in the study: six for *Streptococcus pneumoniae* (6/360 = 1.7%) and one for Human herpesvirus 6 (HHV-6) (1/360 = 0.3%).

For the FilmArray 2.0 system, 365 runs were initiated and 360 runs were completed (98.6% initially valid results). Of the five initially invalid runs, three runs were invalid because they were aborted by the operator due to being run on an incorrect instrument. The two other invalid results were due to a software error (1/365 = 0.3%) and due to an incomplete result caused by a data transfer error (1/365 = 0.3%).

A summary of qualitative results from both reproducibility studies (percent agreement with the expected result) for each analyte and organism concentration is provided in the following tables.

Reproducibility of the FilmArray ME Panel on FilmArray

Organism/Isolate Tested	Concentration	Expected Test Result	FilmArray Agreement with Expected Results			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
BACTERIA						
E. coli K1 b(4))	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 96.7% (96%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
H. influenzae b(4)	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	29/30 96.7%	30/30 100%	30/30 100%	89/90 98.9% (94.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
L. monocytogenes b(4)	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	29/30 96.7%	30/30 100%	30/30 100%	89/90 98.9% (94.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
N. meningitidis	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
S. agalactiae b(4)	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	29/30 96.7%	30/30 100%	27/30 90.0%	86/90 95.6% (89.0%-98.8%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	26/30 86.7%	30/30 100%	27/30 90.0%	83/90 92.2% (84.6%-96.8%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
S. pneumoniae	Negative (No analyte)	Not Detected	118/120 98.3%	118/120 98.3%	118/120 98.3%	354/360[a] 98.3% (96.4.0%-99.4%)
VIRUSES/YEAST						
CMV	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)

Organism/Isolate Tested	Concentration	Expected Test Result	FilmArray Agreement with Expected Results			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
EV Coxsackievirus A9 b(4)	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%	28/30 93.3%	88/90 97.8% (92.2%-99.7%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
HSV-1	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
HSV-2 b(4)	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%	30/30 100%	90/90 100% (96.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
HHV-6	Negative (No analyte)	Not Detected	120/120 100%	119/120 99.2%	120/120 100%	359/360 99.7% (98.5%-100%)
HPeV b(4)	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	29/30 96.7%	30/30 100%	30/30 100%	89/90 98.9% (94.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
VZV b(4)	Moderate Positive 3× LoD 0.3 $TCID_{50}$/mL	Detected	29/30 96.7%	30/30 100%	30/30 100%	89/90 98.9% (94.0%-100%)
	Low Positive 1× LoD 0.1 $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
C. gattii[b] b(4)	Moderate Positive 3×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Low Positive 1×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 93.3%	90/90 100% (96.0%-100%)

Organism/Isolate Tested	Concentration	Expected Test Result	FilmArray Agreement with Expected Results			
			Site A	Site B	Site C	All Sites (95% Confidence Interval)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100 %	180/180 100% (98.0%-100%)

[a]Six false positive *S. pneumoniae* results were reported. The unexpected results were observed at all three test sites, in different samples, on different days, and with different pouch lots. The overall incidence of false *S. pneumoniae* results observed in all reproducibility testing was <1%.
[b]*C. gattii* was tested at concentrations equivalent to 10× and 30× LoD for the combined *Cryptococcus neoformans/gattii* test result on the FilmArray.

Reproducibility of the FilmArray ME Panel on FilmArray 2.0

Organism/Isolate Tested	Concentration	Expected Test Result	FilmArray 2.0 Agreement with Expected Results			
			System A	System B	System C	All Systems (95% Confidence Interval)
BACTERIA						
E. coli K1 b(4))	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	29/30 96.7%	29/30 96.7%	29/30 96.7%	87/90 96.7% (90.6%-99.3%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
H. influenzae b(4)	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
L. monocytogenes b(4)	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
N. meningitidis	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
S. agalactiae b(4)	Moderate Positive 3× LoD 3×10^3 CFU/mL	Detected	29/30 96.7%	30/30 100%	29/30 96.7%	88/90 97.8% (92.2%-99.7%)

Organism/Isolate Tested	Concentration	Expected Test Result	FilmArray 2.0 Agreement with Expected Results			
			System A	System B	System C	All Systems (95% Confidence Interval)
	Low Positive 1× LoD 1×10^3 CFU/mL	Detected	29/30 96.7%	29/30 96.7%	30/30 100%	88/90 97.8% (92.2%-99.7%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
S. pneumoniae	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
VIRUSES/YEAST						
CMV	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
EV Coxsackievirus A9 b(4)	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%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
HSV-1	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
HSV-2 b(4)	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%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
HHV-6	Negative (No analyte)	Not Detected	120/120 100%	120/120 100%	120/120 100%	360/360 100% (99.0%-100%)
HPeV b(4)	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%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
VZV b(4)	Moderate Positive 3× LoD 0.3 $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	29/30 96.7%	89/90 98.9% (94.0%-100%)

Organism/Isolate Tested	Concentration	Expected Test Result	FilmArray 2.0 Agreement with Expected Results			
			System A	System B	System C	All Systems (95% Confidence Interval)
	Low Positive 1× LoD 0.1 $TCID_{50}$/mL	Detected	30/30 100%	30/30 100%	30/30 100%	90/90 100% (96.0%-100%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100%	180/180 100% (98.0%-100%)
C. gattii b(4)	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%)
	Negative (No analyte)	Not Detected	60/60 100%	60/60 100%	60/60 100 %	180/180 100% (98.0%-100%)

The following table includes analyses of assay reproducibility by analyte and panel member based on the Tm for each positive result observed during both the FilmArray system and FilmArray 2.0 system Reproducibility studies. Results demonstrated similar performance for both instruments with Tm standard deviations of ≤ 0.5°C for all analytes and concentrations evaluated.

Reproducibility of Tm values for Positive FilmArray ME Assays on FilmArray and FilmArray 2.0 systems

Test Result (Organism/Isolate Tested)	Assay	Concentration Tested (× LoD)	Mean Tm (°C) (±StDev)							
			FilmArray				FilmArray 2.0			
			Site A	Site B	Site C	All Sites	System A	System B	System C	All Systems
RNA Process Control	yeastRNA		82.4 **±0.2**	82.5 **±0.3**	82.0 **±0.2**	82.3 **±0.3**	81.7 **±0.3**	81.9 **±0.3**	81.7 **±0.3**	81.8 **±0.3**
PCR2 Control	PCR2		76.5 **±0.1**	76.6 **±0.3**	76.1 **±0.2**	76.4 **±0.3**	75.5 **±0.3**	75.7 **±0.3**	75.5 **±0.3**	75.6 **±0.3**
BACTERIA										
***E. coli* K1** b(4)	Ecoli 3	**3× LoD**	82.9 **± 0.1**	82.8 **± 0.3**	82.6 **± 0.1**	82.8 **±0.3**	82.1 **±0.3**	82.3 **±0.3**	82.1 **±0.3**	82.2 **±0.3**
		1× LoD	83.0 **±0.1**	83.1 **±0.3**	82.6 **±0.2**	82.9 **± 0.3**	82.2 **±0.3**	82.4 **±0.3**	82.3 **±0.3**	82.3 **±0.3**
H. influenzae b(4) b(4)	Hinfluenzae 1	**3× LoD**	78.6 **±0.1**	78.6 **±0.4**	78.2 **±0.2**	78.5 **±0.4**	77.9 **±0.3**	77.9 **±0.3**	77.7 **±0.2**	77.8 **±0.3**
		1× LoD	78.6 **±0.2**	78.7 **±0.3**	78.3 **±0.2**	78.5 **± 0.3**	78.0 **±0.3**	78.0 **±0.3**	77.9 **±0.3**	78.0 **±0.3**
	Hinfluenzae 2	**3× LoD**	82.0 **±0.1**	81.9 **±0.4**	81.6 **±0.2**	81.8 **± 0.3**	81.0 **±0.2**	81.1 **±0.2**	81.0 **±0.2**	81.0 **±0.2**
		1× LoD	82.0 **±0.1**	82.0 **±0.3**	81.7 **±0.2**	81.9 **± 0.3**	81.2 **±0.2**	81.3 **±0.2**	81.2 **±0.3**	81.2 **±0.2**
L. monocytogenes (b(4) b(4)	Lmonocytogenes	**3× LoD**	80.4 **±0.2**	80.6 **±0.3**	80.2 **±0.2**	80.4 **± 0.3**	80.0 **±0.4**	80.1 **±0.3**	80.0 **±0.3**	80.0 **±0.3**
		1× LoD	80.5 **±0.2**	80.6 **±0.4**	80.1 **±0.2**	80.4 **±0.4**	80.0 **±0.4**	80.1 **±0.3**	79.9 **±0.2**	80.0 **±0.3**
S. agalactiae b(4) b(4)	Sagalactiae	**3× LoD**	82.0 **±0.1**	82.0 **±0.3**	81.6 **±0.2**	81.9 **± 0.3**	81.1 **±0.3**	81.3 **±0.3**	81.2 **±0.2**	81.2 **±0.3**
		1× LoD	82.1	82.2	81.7	82.0	81.2	81.4	81.2	81.3

Test Result (Organism/Isolate Tested)	Assay	Concentration Tested (× LoD)	Mean Tm (°C) (±StDev)							
			FilmArray				FilmArray 2.0			
			Site A	Site B	Site C	All Sites	System A	System B	System C	All Systems
			±0.1	±0.3	±0.2	± 0.3	±0.3	±0.2	±0.2	±0.3
VIRUSES										
EV (Coxsackievirus A9) b(4))	EV2	**3× LoD**	89.7	89.7	89.3	89.6	89.1	89.3	89.2	89.2
			±0.1	±0.3	±0.2	± 0.3	±0.3	±0.3	±0.3	±0.3
		1× LoD	89.7	89.7	89.3	89.6	89.1	89.3	89.2	89.2
			±0.1	±0.4	±0.2	± 0.3	±0.3	±0.3	±0.2	±0.3
HSV-2 b(4))	HSV2 1	**3× LoD**	75.6	75.5	75.1	75.4	74.6	74.8	74.6	74.7
			±0.3	±0.3	±0.3	± 0.3	±0.3	±0.3	±0.3	±0.3
		1× LoD	75.9	76.0	75.4	75.7	74.9	75.1	74.9	75.0
			±0.2	±0.2	±0.3	±0.4	±0.3	±0.3	±0.3	±0.3
	HSV2 2	**3× LoD**	88.8	88.9	88.4	88.7	88.1	88.3	88.2	88.2
			±0.2	±0.4	±0.2	± 0.3	±0.3	±0.3	±0.2	±0.3
		1× LoD	88.9	89.0	88.5	88.8	88.2	88.5	88.3	88.3
			±0.2	±0.3	±0.2	±0.4	±0.3	±0.3	±0.2	±0.3
HPeV b(4))	HPeV	**3× LoD**	82.8	82.8	82.5	82.7	82.2	82.3	82.1	82.2
			±0.2	±0.4	±0.2	± 0.3	±0.3	±0.3	±0.2	±0.3
		1× LoD	82.8	82.9	82.5	82.7	82.3	82.3	82.2	82.3
			±0.2	±0.3	±0.2	± 0.3	±0.3	±0.2	±0.3	±0.3
VZV b(4)	VZV 1	**3× LoD**	88.9	89.0	88.5	88.8	88.5	88.5	88.4	88.5
			±0.2	±0.4	±0.2	±0.4	±0.4	±0.2	±0.2	±0.3
		1× LoD	89.0	89.0	88.5	88.8	88.5	88.5	88.3	88.4
			±0.2	±0.5	±0.2	±0.4	±0.3	±0.3	±0.2	±0.3
	VZV 2	**3× LoD**	82.0	82.1	81.7	81.9	81.5	81.5	81.4	81.5
			±0.1	±0.3	±0.2	± 0.3	±0.3	±0.2	±0.2	±0.2
		1× LoD	82.1	82.1	81.7	82.0	81.5	81.5	81.4	81.5
			±0.1	±0.4	±0.2	± 0.3	±0.3	±0.2	±0.2	±0.2
YEAST										
C. neoformans/gattii (C. gattii) b(4)	Cryptococcus	**30× LoD[a] (FilmArray)**	82.0	82.0	81.6	81.8	81.2	81.4	81.3	81.3
		3× LoD (FilmArray 2.0)	±0.1	±0.3	±0.2	± 0.3	±0.3	±0.3	±0.3	±0.3
		10× LoD[a] (FilmArray)	82.0	82.1	81.6	81.9	81.3	81.5	81.3	81.4
		1× LoD (FilmArray 2.0)	±0.2	±0.2	±0.2	±0.3	±0.3	±0.4	±0.2	±0.3

[a] *C. gattii* was tested at concentrations equivalent to 10× and 30× LoD for the combined *Cryptococcus neoformans/gattii* test result on the FilmArray system, while *C. gattii* was tested at the intended 1× and 3× LoD concentrations on the FilmArray 2.0 system.

b. Linearity/assay Reportable Range:

Not Applicable

c. Traceability, Stability, Expected Values (controls, calibrators, or methods):

Internal Controls:

Two internal controls are included in each FilmArray ME Panel pouch:

- RNA Process Control: The RNA Process Control assay targets an RNA transcript from the yeast *Schizosaccharomyces pombe*. b(4) . The control material is carried through all stages of the test process, including lysis, nucleic acid purification, reverse transcription, 1st stage PCR, dilution, 2nd stage PCR and DNA melting. A positive control result indicates that all steps carried out in the FilmArray ME Panel pouch were successful.

- PCR2 Control: The PCR2 Control assay detects a DNA target b(4) . A positive result indicates that the 2nd stage PCR was successful.

Both internal control assays must be positive for the test run to pass. When either control fails, the Controls field of the test report will display "Failed" and all results will be listed as Invalid. If the controls fail, the user is instructed to repeat the test using a new pouch.

Of the six pouch control failures observed in the prospective clinical study, five were attributed to both RNA Process Control and PCR 2 Control failures and one was attributed to RNA Process Control failure only.

Recommended External Controls:

External controls are not provided with the FilmArray ME Panel, but are recommended in the package insert. Molecular grade water or artificial CSF can be used as an external negative control. Previously characterized CSF specimens or negative matrix spiked with well-characterized organisms can be used as external positive controls. External controls should be used in accordance with the appropriate accrediting organization requirements, as applicable.

d. Detection Limit:

Evaluation of the Limit of Detection:

The limit of detection (LoD) for FilmArray ME Panel analytes was estimated by testing dilutions of contrived samples containing known concentrations of bacteria, viruses or yeast detected by the FilmArray ME Panel. Representative strains were chosen in order to obtain positive results for every assay on the panel and multiple strains were evaluated to cover clinically important species or variants for some analytes.

Samples for LoD testing were prepared in aCSF (artificial CSF obtained from a commercial provider) matrix and contained one or up to five targeted organisms. b(4) . b(4) The LoD was confirmed for all analytes on both FilmArray and FilmArray 2.0 systems. Data presented in the following table are from LoD confirmation testing on the FilmArray system.

Limit of Detection Results for FilmArray ME Panel Analytes

ME Panel Test Result	Species/Isolate Tested	LoD Concentration	Detection at LoD Concentration
BACTERIA			
***E. coli* K1**	*E. coli* K1, strain C5 b(4) b(4)	1×10^3 CFU/mL	20/20 100%
H. influenzae	*H. influenzae*, strain AMC 36-A-1 b(4)	1×10^3 CFU/mL	20/20 100%
L. monocytogenes	*L. monocytogenes*, strain 1071/53, b(4)	1×10^3 CFU/mL	20/20 100%
N. meningitidis	*N. meningitidis*, strain M-1574 b(4)	100 CFU/mL	19/20 95%
S. agalactiae	*S. agalactiae*, type strain, G19, group B b(4)	1×10^3 CFU/mL	20/20 100%
S. pneumoniae	*S. pneumoniae*, strain SV 1, serotype 1 b(4)	100 cells/mL	19/20 95%
VIRUSES			
CMV	CMV, strain AD-169 b(4)	100 $TCID_{50}$/mL (4.30×10^3 copies/mL)	20/20 100%
EV (Species A-D)	Coxsackievirus A6, species A, b(4)	50 $TCID_{50}$/mL	20/20 100%
	Coxsackievirus A9, species B b(4)	5 $TCID_{50}$/mL	20/20 100%
	Coxsackievirus A17, species C, strain G-12 b(4)	5 $TCID_{50}$/mL	20/20 100%
	EV 70, species D, b(4) b(4)	50 $TCID_{50}$/mL	20/20 100%
HSV-1	HSV-1, strain MacIntyre b(4)	250 $TCID_{50}$/mL (1.51×10^3 copies/mL)	20/20 100%
HSV-2	HSV-2, strain MS b(4)	50 $TCID_{50}$/mL (1.29×10^3 copies/mL)	20/20 100%
HHV-6	HHV-6A, strain U1102 b(4)	1×10^4 copies/mL	19/20 95%
	HHV-6B, strain HST b(4)	1×10^4 copies/mL	19/20 95%
HPeV	HPeV, type 3 b(4)	500 $TCID_{50}$/mL	19/20 95%
VZV	VZV, strain Ellen b(4)	0.10 $TCID_{50}$/mL (1.66×10^3 copies/mL)	20/20 100%
YEAST			

ME Panel Test Result	Species/Isolate Tested	LoD Concentration	Detection at LoD Concentration
C. neoformans/gattii	*C. neoformans* var. *grubii*, type b(4)	100 CFU/mL	20/20 100%
	C. gattii, strain A6MR38, b(4)	100 CFU/mL	20/20 100%

Evaluation of Effect on Analyte LoDs in Multi-Spiked Samples:

A study was performed to determine the applicability of evaluating multiple analytes in a single CSF sample during the analytical studies and evaluated for potential loss of detection when multiple targets are present in clinical specimens (co-infections). Samples consisting of three different organism mixes with up to five targeted organisms each were compared to contrived single-spike samples for the following representative panel analytes: bacterium (*E. coli* K1), yeast (*C. neoformans*), DNA virus (HSV-1), and RNA virus (HPeV). All samples were prepared in aCSF matrix. Serial dilutions were prepared for both single-spike samples and multiple spiked samples containing the corresponding analyte for comparison.

For *E. coli* K1 and HSV-1 samples prepared with analyte concentrations at LoD, 4/4 replicates were positive for both single and multi-spiked samples. Dilutions with concentrations below LoD showed a loss of detection between the single-spiked and multi-spiked specimens at the same organism concentration, thus demonstrating no difference in assay detection for *E. coli* K1 and HSV-1 for single versus multi-spiked samples.

For *C. neoformans*, 4/4 samples were positive at both 1x LoD and 0.1× LoD. At 0.01× LoD, 4/4 were positive for single-spiked samples and 1/4 replicates were positive for multi-spiked samples. To evaluate whether the observed difference in detection was due to multi-spiking versus single-spiking, mean PCR crossing point (Cp) values were evaluated for each concentration evaluated. The analysis showed that mean Cp values for each concentration tested showed no trend toward lower or higher Cp values between single and multi-spiked samples.

For HPeV at 1× LoD, 3/4 replicates were detected for multi-spiked samples and 4/4 replicates were detected for single-spiked samples. However, at two different dilutions below the LoD, there was no trend in detection differences between single and multi-spiked samples.

In summary, testing of single versus multi-spiked samples did not show a significant difference in assay performance for the four representative analytes evaluated. The study results supported the use of multi-spiked samples in various analytical studies.

e. Analytical Reactivity (Inclusivity):

Inclusivity testing was performed using contrived samples consisting of 96 isolates

spiked into artificial CSF (aCSF) sample matrix at a concentration near the LoD for each analyte (1× to 3× LoD). Strains were selected to represent relevant species, subspecies, or serotypes. For any isolate that was not detected at the initial test concentration, results were reviewed and if reactivity was expected, the isolate was retested at the same concentration up to 5 times. If needed, retesting of the isolate was also performed at a higher concentration (typically 10× LoD) and a detected result was a demonstration of reactivity with the isolate at the elevated concentration.

The FilmArray ME panel gave positive results for the majority of isolates evaluated when tested at sample concentrations near the LoD (1× to 3× LoD). One strain of HpeV (Serotype 5) was detected at 10× LoD as compared to the HpeV strain evaluated in the LoD study.

When possible, *in silico* analysis of sequence data was used to make predictions of assay reactivity for less common strains or serotypes that were not evaluated with empirical testing.

The following tables includes a summary of ME Panel reactivity based on empirical data with footnotes describing predictions of assay reactivity based on *in silico* analysis.

Summary of FilmArray ME Panel Analytical Reactivity (Inclusivity)

FilmArray ME Panel Test Result	# of Isolates Tested and Detected	Concentration Detected	Isolates Tested and Detected
Bacteria			
E. coli K1	5	1,000 - 3,000 CFU/mL	*E. coli* strains of the K1 serotype only
H. influenzae	9	1,000 - 3,000 CFU/mL	Non-typeable and typeable (types a-f) strains of *H. influenzae*
L. monocytogenes	6	1,000 - 3,000 CFU/mL	Types 1/2a, 1/2b, and 4b of *L. monocytogenes*[a]
N. meningitidis	7	100 - 300 CFU/mL	Encapsulated *N. meningitidis* (serotypes W135, A, B, C, D, Y and DNA from a strains with a variant *ctrA* gene)
S. agalactiae	5	1,000 - 3,000 CFU/mL	Multiple serotypes or isolates of *S. agalactiae* (Group B *Streptococcus*)
S. pneumoniae	6	100 - 300 cells/mL	Multiple serotypes of *S. pneumoniae*
Viruses			
CMV	5	100 - 300 $TCID_{50}$/mL (4.3×10^3 - 1.3×10^4 copies/mL)	Multiple strains of Cytomegalovirus (CMV).
EV	18	5 - 50 $TCID_{50}$/mL	Representative isolates from all species (A-D) and several serotypes of human Enterovirus, Coxsackievirus, and Echovirus[b]
HSV-1	5	250 - 750 $TCID_{50}$/mL (1.5×10^3 - 4.5×10^3 copies/mL)	Multiple strains of Herpes simplex virus 1 (HSV-1)
HSV-2	5	50 - 150 $TCID_{50}$/mL (1.3×10^3 - 3.9×10^3 copies/mL)	Multiple strains of Herpes simplex virus 2 (HSV-2)
HHV-6	4	1×10^4 - 3×10^4 copies/mL	A and B variants of Human herpesvirus 6 (HHV-6)

FilmArray ME Panel Test Result	# of Isolates Tested and Detected	Concentration Detected	Isolates Tested and Detected
HPeV	6	500 - 5,000 $TCID_{50}$/mL	Serotypes 1-6 of Human parechovirus (HPeV)[c]
VZV	5	0.1 - 0.3 $TCID_{50}$/mL (1.7×10^3- 5×10^3 copies/mL)	Multiple strains of Varicella zoster virus (VZV)
Yeast			
C. neoformans/gattii	10 (5 per species)	100 - 300 CFU/mL	Multiple strains, serotypes, and genotypes of *Cryptococcus neoformans* and *Cryptococcus gattii*

[a] *In silico* analysis of available sequences predicts that the FilmArray ME Panel will react with all currently characterized strains and serotypes of *L. monocytogenes*.
[b] *In silico* analysis of available sequences predicts that the FilmArray ME Panel will react with all currently characterized serotypes (>100) of human enteroviruses (including enteroviruses, coxsackieviruses, and echoviruses).
[c] Based on sequence analysis, the FilmArray ME Panel is also predicted to react with HPeV serotypes 7 and 8. No sequence data were available for predicting reactivity with other serotypes.

The following tables include specific strains evaluated with the FilmArray ME Panel.

Results for *Escherichia coli* K1 Inclusivity Testing

Organism	Isolate ID	Serotype [Strain-Year Isolated]	Concentration Tested	Test Result
Escherichia coli K1	b(4)	Serotype O18ac:K1:H7 [Strain C5 [Bort]-1975]	1×10^3 CFU/mL (1× LoD)	Detected
		Serotype O2:K1:H4 [Strain U9-41]	3×10^3 CFU/mL	Detected
		Serotype O16:K1:H- [Strain F11119-41-1952]	3×10^3 CFU/mL	Detected
		Serotype O9:K1:H- [Strain Bi 7509/41-1952]	3×10^3 CFU/mL	Detected
		Serotype O45:K1:H10 [Strain H61-1952]	3×10^3 CFU/mL	Detected

Results for *Haemophilus influenzae* Inclusivity Testing

Organism	Isolate ID	Type [Strain]	Concentration Tested	Test Result
Haemophilus influenzae	b(4)	Non-typeable [strain Rd [KW20]]	3×10^3 CFU/mL	Detected
		Non-typeable biogroup aegyptius [type strain, 180-a]	3×10^3 CFU/mL	Detected
		Type a [strain AMC 36-A-3]	3×10^3 CFU/mL	Detected
		Type b [strain Rab]	3×10^3 CFU/mL	Detected
		Type b [biotype 1]	1×10^3 CFU/mL (1× LoD)	Detected
		Type c [strain C 9007]	3×10^3 CFU/mL	Detected

Organism	Isolate ID	Type [Strain]	Concentration Tested	Test Result
	b(4)	Type d [strain AMC 36-A-6]	3×10^3 CFU/mL	Detected
		Type e [strain AMC 36-A-7]	3×10^3 CFU/mL	Detected
		Type f [strain GA-1264]	3×10^3 CFU/mL	Detected

Results for *Listeria monocytogenes* Inclusivity Testing

Organism	Source	Isolate ID	Type	Concentration Tested	Test Result
Listeria monocytogenes	b(4)		Type 1/2a	3×10^3 CFU/mL	Detected
			Type 1/2a	3×10^3 CFU/mL	Detected
			Type 1/2b	3×10^3 CFU/mL	Detected
			Type 1/2b	3×10^3 CFU/mL	Detected
			Type 4b	3×10^3 CFU/mL	Detected
			Type 4b	1×10^3 CFU/mL (1× LoD)	Detected

Note: At least 12 serotypes of *L. monocytogenes* have been recognized (i.e., 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4b, 4c, 4d, 4e and 7) based on somatic (O) and flagellar (H) antigens, but more than 90% of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. *In silico* analysis based on available sequence data suggest that all serotypes are expected to be amplified by the assay and detected by the FilmArray ME Panel.

Results for *Neisseria meningitidis* Inclusivity Testing

Organism	Isolate ID	Serotype	Concentration Tested	Test Result
Neisseria meningitidis (Encapsulated)	b(4)	Serotype W135	100 CFU/mL (1× LoD) [~1.9×10^3 copies/mL]	Detected
		Serotype A	300 CFU/mL	Detected
		Serotype B	300 CFU/mL	Detected
		Serotype C	300 CFU/mL	Detected
		Serotype D	300 CFU/mL	Detected
		Serotype Y	300 CFU/mL	Detected
		DNA with variant *ctrA* gene	5.6×10^3 copies/mL (~3× LoD)	Detected

Results for *Streptococcus agalactiae* Inclusivity Testing

Organism	Isolate ID	Serotype	Concentration Tested	Test Result
Streptococcus agalactiae	b(4)	Serotype I a/c Type strain	1×10^3 CFU/mL (1× LoD)	Detected
		Serotype III	3×10^3 CFU/mL	Detected
		Serotype V	3×10^3 CFU/mL	Detected
		Unknown	3×10^3 CFU/mL	Detected
		Unknown	3×10^3 CFU/mL	Detected

Note: *In silico* analysis predicts that the FilmArray ME panel should detect *S. agalactiae* serotypes Ia, Ia/c, Ib, II, III, V and VIII strains.

Results for *Streptococcus pneumoniae* Inclusivity Testing

Organism	Isolate ID	Serotype	Concentration Tested	Test Result
Streptococcus pneumoniae	b(4)	Serotype 1	100 cells/mL (1× LoD)	Detected
		Serotype 4	300 cells/mL	Detected
		Serotype 5	300 cells/mL	Detected
		Serotype 11A	300 cells/mL	Detected
		Serotype 14	300 cells/mL	Detected
		Serotype 19A	300 cells/mL	Detected

Note: Based on the serotype information associated with sequences available in public databases, *in silico* analysis indicates the assay will react with all serotypes of *S. pneumoniae*, including those covered by the PPSV23 pneumococcal vaccine (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F).

Results for Cytomegalovirus (CMV) Inclusivity Testing

Organism	Isolate ID	Strain	Location/ Year Isolated	Concentration Tested	Test Result
Cytomegalovirus (CMV) [Human Herpesvirus 5]	b(4)	AD-169	unknown	100 $TCID_{50}$/mL [4.3×10^3 copies/mL] (1× LoD)	Detected
		Towne	Unknown	1.3×10^4 copies/mL	Detected
		Merlin	Wales, 2003	1.3×10^4 copies/mL	Detected

Organism	Isolate ID	Strain	Location/ Year Isolated	Concentration Tested	Test Result
	b(4)	Davis	1957	1.3×10^4 copies/mL	Detected
		Toledo	Virginia, USA 2011	1.3×10^4 copies/mL	Detected

Results for Enterovirus (EV) Inclusivity Testing

Species	Virus/Serotype		Strain Location/Year	Concentration Tested	Test Result
A	Coxsackievirus A6	b(4)	Gdula b(4)	50 $TCID_{50}$/mL (1× LoD)	Detected
	Coxsackievirus A10		M.K. (Kowalik) b(4)	150 $TCID_{50}$/mL	Detected
				50 $TCID_{50}$/mL	Detected
	Coxsackievirus A16		H07314 334	150 $TCID_{50}$/mL	Detected
				50 $TCID_{50}$/mL	Detected
	Enterovirus 71		BrCr	150 $TCID_{50}$/mL	Detected
				50 $TCID_{50}$/mL	Detected
B	Coxsackievirus A9		-	5 $TCID_{50}$/mL (1× LoD)	Detected
	Coxsackievirus B1		H07248 372	15 $TCID_{50}$/mL	**Not Detected**
				50 $TCID_{50}$/mL	Detected
	Coxsackievirus B2		H08084 367	15 $TCID_{50}$/mL	**Not Detected**
				50 $TCID_{50}$/mL	Detected
	Coxsackievirus B3		-	15 $TCID_{50}$/mL	Detected
	Coxsackievirus B4		-	15 $TCID_{50}$/mL	Detected
	Coxsackievirus B5		-	15 $TCID_{50}$/mL	**Not Detected**
				50 $TCID_{50}$/mL	Detected
	Echovirus 6		-	15 $TCID_{50}$/mL	**Not Detected**
				50 $TCID_{50}$/mL	Detected
	Echovirus 9		-	15 $TCID_{50}$/mL	Detected
	Echovirus 18		H07218 472	15 $TCID_{50}$/mL	**Not Detected**
				50 $TCID_{50}$/mL	Detected
C	Coxsackievirus A17		G-12 b(4)	5 $TCID_{50}$/mL (1× LoD)	Detected
	Coxsackievirus A21		Kuykendall b(4)	15 $TCID_{50}$/mL	Detected
	Coxsackievirus A24		DN-19 b(4)	15 $TCID_{50}$/mL	Detected
D	Enterovirus 70		J670/71 b(4)	50 $TCID_{50}$/mL (1× LoD)	Detected
	Enterovirus 68 (aka Rhinovirus 87)		-	150 $TCID_{50}$/mL	Detected
				50 $TCID_{50}$/mL	Detected

Note: *In silico* analysis of available sequences predicts that the FilmArray ME Panel will react with all currently characterized serotypes (>100) of human enteroviruses (including enteroviruses, coxsackieviruses, and echoviruses).

Results for Herpes Simplex Virus 1 (HSV-1) Inclusivity Testing

Organism	Isolate ID	Strain	Concentration Tested	Test Result
Herpes simplex virus 1 (HSV-1)	b(4)	MacIntyre	250 $TCID_{50}$/mL [1.5×10^3 copies/mL] (1× LoD)	Detected
		F	4.5×10^3 copies/mL	Detected
		HF	4.5×10^3 copies/mL	Detected
		KOS	4.5×10^3 copies/mL	Detected
		b(4) -2011-1	4.5×10^3 copies/mL	Detected

Results for Herpes Simplex Virus 2 (HSV-2) Inclusivity Testing

Organism	Isolate ID	Strain	Concentration Tested	Test Result
Herpes simplex virus 2 (HSV-2)	b(4)	MS	50 $TCID_{50}$/mL [1.3×10^3 copies/mL] (1× LoD)	Detected
		G	3.9×10^3 copies/mL	Detected
		b(4) -2011-2	3.9×10^3 copies/mL	Detected
		131596	3.9×10^3 copies/mL	Detected
		HG52	3.9×10^3 copies/mL	Detected

Results for Human Herpesvirus 6 (HHV-6) Inclusivity Testing

Organism	Isolate ID	Strain	Concentration Tested	Test Result
Human Herpesvirus 6A	b(4)	U1102	1×10^4 copies/mL (1× LoD)	Detected
Human Herpesvirus 6B		HST	1×10^4 copies/mL (1× LoD)	Detected
		SF	3×10^4 copies/mL	Detected
		Z29	3×10^4 copies/mL	Detected

Results for Human Parechovirus (HPeV) Inclusivity Testing

Organism	Isolate ID	Serotype	Concentration Tested	Test Result
Human Parechovirus (HPeV)	b(4)	Serotype 1	1.5×10^3 $TCID_{50}$/mL	Detected
		Serotype 2	1.5×10^3 $TCID_{50}$/mL	Detected

Organism	Isolate ID	Serotype	Concentration Tested	Test Result
	b(4)	Serotype 3	500 $TCID_{50}$/mL (1× LoD)	Detected
		Serotype 4	1.5×10^3 $TCID_{50}$/mL	Detected
		Serotype 5	1.5×10^3 $TCID_{50}$/mL	**Not Detected**
			5×10^3 $TCID_{50}$/mL (10× LoD)	Detected
		Serotype 6	1.5×10^3 $TCID_{50}$/mL	Detected

Note: *In silico* analysis predicts detection of all serotypes of HPeV for which there are available sequences in the database (serotypes 1-8).

Results for Varicella Zoster Virus (VZV) Inclusivity Testing

Organism	Isolate ID	Strain	Concentration Tested	Test Result
Varicella Zoster Virus (Human Herpesvirus 3)	b(4)	Ellen b(4)b(4) VR-1367)	0.10 $TCID_{50}$/mL [1.7×10^3 copies/mL] (1× LoD)	Detected
		Isolate A	5×10^3 copies/mL	Detected
		Isolate B	5×10^3 copies/mL	Detected
		Strain 275	5×10^3 copies/mL	Detected
		Webster	5×10^3 copies/mL	Detected

Results for *Cryptococcus neoformans/gattii* Inclusivity Testing

Organism	Isolate ID	Serotype/Strain Info	Isolate Location	Concentration Tested	Test Result
Cryptococcus neoformans	b(4)	type strain, CBS 132	b(4)	300 CFU/mL	Detected
		Serotype A, strain H99 type strain of var. *grubii*		100 CFU/mL (1× LoD)	Detected
		Serotype A strain WM148, type VNI		300 CFU/mL	Detected
		Serotype AD strain WM628, type VNIII		300 CFU/mL	Detected
		Serotype D strain WM629, type VNIV		300 CFU/mL	Detected
Cryptococcus gattii		Serotype B strain WM179, type VGI		300 CFU/mL	Detected
		Serotype B strain R272, type VGIIb		300 CFU/mL	Detected
		Unknown serotype strain R38, type VGIIc		100 CFU/mL (1× LoD)	Detected
		Serotype B strain WM161, type VGIII		300 CFU/mL	Detected

Organism	Isolate ID	Serotype/Strain Info	Isolate Location	Concentration Tested	Test Result
	b(4) b(4)	Serotype C strain WM779, type VGIV	b(4) a	300 CFU/mL	Detected

f. Competitive Inhibition/Microbial Interference Studies:

Potentially competing or interfering viruses and other microorganisms were evaluated for their effect on FilmArray ME Panel performance.

To evaluate the potential for competitive inhibition between on-panel analytes (detected by FilmArray ME Panel), two sample mixes were prepared in aCSF matrix using 10 representative FilmArray ME Panel organisms at concentrations equivalent to approximately 3× LoD for each analyte. Samples were co-spiked with high concentrations of four representative ME Panel organisms (*E. coli*, Coxackievirus A9, HSV-1, and *C. neoformans*).

To evaluate the potential for interference from off-panel organisms (not detected by the FilmArray ME Panel), the same two sample mixes comprised of 10 FilmArray ME organisms spiked at approximately 3× LoD were co-spiked with high concentrations of off-panel organisms.

All FilmArray ME analytes were detected as expected and therefore the study results demonstrated no competitive inhibition or microbial interference from high concentrations of on-panel or off-panel organisms. The organisms evaluated for potential inhibition/interference are presented in the following table.

Organisms Evaluated for Competitive Inhibition/Microbial Interference

Competitive Inhibition	Concentration Tested	Results
Escherichia coli (K1)	1.02×10^{8} CFU/mL	No Inhibition/Interference
Coxsackievirus A9 (Enterovirus)	2.19×10^{5} $TCID_{50}$/mL	No Inhibition/Interference
Herpes simplex virus 1	1.95×10^{6} $TCID_{50}$/mL	No Inhibition/Interference
Cryptococcus neoformans	8.10×10^{5} CFU/mL	No Inhibition/Interference
Viral or Microbial Interference	**Concentration Tested**	**Results**
Epstein-Barr virus	1.64×10^{9} $TCID_{50}$/mL	No Interference
Influenza A H1N1-2009	2.45×10^{4} $TCID_{50}$/mL	No Interference
Proprionibacterium acnes	1.12×10^{7} cells/mL	No Interference
Staphylococcus epidermidis	1.95×10^{7} CFU/mL	No Interference
Escherichia coli (non-K1)	1.38×10^{8} CFU/mL	No Interference
Staphylococcus aureus	8.55×10^{6} CFU/mL	No Interference
Candida albicans	1.01×10^{6} CFU/mL	No Interference

g. Analytical Specificity/Cross-reactivity:

Potential cross-reactivity for the FilmArray ME Panel was evaluated by testing high concentrations of organisms in contrived aCSF samples. The strains tested consisted of 19 on-panel (organisms identified by FilmArray ME Panel) and 107 off-panel organisms.

Samples were prepared in aCSF matrix at the highest concentration possible based on the available material, with most samples containing analyte concentrations of at least 10^6 CFU/mL for bacteria, 10^4 units/mL for viruses and 10^5 CFU/mL for yeast and protists.

No inter-assay cross-reactivity was observed for the 19 on-panel organisms evaluated in the study.

On Panel Organisms/Viruses Evaluated for Cross-Reactivity

Organism	Isolate ID	Organism	Isolate ID
Escherichia coli K1	b(4)	Enterovirus (Enterovirus 70)	b(4)
Haemophilus influenzae		Herpes simplex virus 1	
Listeria monocytogenes		Herpes simplex virus 2	
Neisseria meningitidis		Human herpesvirus 6A	
Streptococcus agalactiae		Human herpesvirus 6B	
Streptococcus pneumoniae		Human parechovirus	
Cytomegalovirus		Varicella zoster virus	
Enterovirus (Coxsackievirus A6)		*Cryptococcus neoformans*	
Enterovirus (Coxsackievirus A9)		*Cryptococcus gattii*	
Enterovirus (Coxsackievirus A17)			

Off-panel organisms evaluated in the cross-reactivity study were selected based on their clinical relevance, their likelihood of being present in CSF and/or their genetic similarity to FilmArray ME Panel assay sequences as determined by *in silico* analysis.

As was also predicted by *in silico* analysis, study results demonstrated cross-reactivity with *H. haemolyticus* (positive for *H. influenzae*) and Rhinoviruses (positive for Enterovirus). Cross-reactivity was also observed with *Cryptococcus amylolentus* (positive for *C. neoformans*/*gattii*), a near-neighbor to *Cryptococcus neoformans* that does not infect humans. No other cross-reactivity was predicted or observed. The following table lists the off-panel bacteria, viruses, fungi, and protists that were evaluated with the FilmArray ME Panel for this study. Organisms that demonstrated cross-reactivity are in bold.

Off-Panel Organisms Evaluated for Cross-Reactivity

Gram-positive Bacteria	Gram-negative Bacteria	Viruses	
Bacillus cereus	*Citrobacter freundii*	Adenovirus A12	Parainfluenza virus 2
Corynebacterium striatum	*Cronobacter sakazakii*	Adenovirus C2	Parainfluenza virus 4
Corynebacterium urealyticus	*Enterobacter aerogenes*	Adenovirus D20	Parvovirus B19
Listeria ivanovii	*Enterobacter cloacae*	Adenovirus E4	Respiratory Syncytial Virus
Listeria innocua	*Escherichia coli* (non-K1)	Adenovirus F41	Rotavirus
Mycobacterium tuberculosis	*Escherichia fergusonii*	BK polyoma virus	Rubella Virus
Proprionibacterium acnes	*Escherichia hermanii*	Coronavirus 229E	St. Louis Encephalitis Virus
Staphylococcus aureus	*Escherichia vulneris*	Coronavirus NL63	West Nile Virus
Staphylococcus capitis	*Haemophilus ducreyi*	Coronavirus OC43	**Fungi**
Staphylococcus epidermidis	***Haemophilus haemolyticus***[a]	Dengue virus (Type 2)	*Aspergillus fumigatus*
Staphylococcus haemolyticus	*Haemophilus parahaemolyticus*	Epstein-Barr Virus	*Candida albicans*
Staphylococcus hominis	*Haemophilus parainfluenzae*	Hepatitis B virus (HBV)	*Candida krusei*
Staphylococcus lugdunensis	*Klebsiella pneumoniae*	Hepatitis C virus (HCV)	*Candida parapsilosis*
Staphylococcus saprophyticus	*Morganella morganii*	Human herpesvirus 7	*Candida tropicalis*
Streptococcus anginosus	*Neisseria meningitidis (Unencapsulated)*	Human herpesvirus 8	*Cryptococcus albidus*
Streptococcus bovis	*Neisseria gonorrhoeae*	Human Immunodeficiency Virus	***Cryptococcus amylolentus***[c]
Streptococcus dysgalactiae	*Neisseria lactamica*	**Human Rhinovirus A1**[b]	*Cryptococcus laurentii*
Streptococcus intermedius	*Neisseria mucosa*	**Human Rhinovirus A16**[b]	*Cryptococcus uniguttulatus*
Streptococcus mitis (tigurinus)	*Neisseria sicca*	**Human Rhinovirus B3**[b]	*Filobasidium capsuligenum*
Streptococcus mutans	*Pantoea agglomerans*	**Human Rhinovirus B83**[b]	**Pathogenic Protists**
Streptococcus oralis	*Proteus mirabilis*	Influenza A H1N1	*Naeglaria fowleri*
Streptococcus pseudopneumoniae	*Pseudomonas aeruginosa*	Influenza A H1N1-2009	*Toxoplasma gondii*
Streptococcus pyogenes	*Salmonella bongori*	Influenza A H3N2	
Streptococcus salivarius	*Salmonella enterica*	Influenza B	
Streptococcus sanguinis	*Serratia marcescens*	JC polyoma virus	
	Shigella boydii	La Crosse Encephalitis Virus	
	Shigella flexneri	Measles Virus	
	Shigella sonnei	Mumps Virus	
	Treponema pallidum		

[a] Detected by the FilmArray ME Panel as *Haemophilus influenzae*. *H. haemolyticus* is a commensal bacterium of the upper respiratory tract, rarely isolated from CSF. Cross-reactivity was observed only at concentrations > 1×10^5 CFU/mL.
[b] Detected by the FilmArray ME Panel as Enterovirus. Human Rhinoviruses are respiratory pathogens and rarely isolated from CSF.
[c] Detected by the FilmArray ME Panel as *Cryptococcus neoformans/gattii*. *C. amylolentus* is not isolated from humans (normal habitat is insect frass).

h. Interfering Substances

A study was performed to evaluate the FilmArray ME Panel for potential interference with the assay from substances that could be present in CSF specimens at the time of collection or introduced into CSF specimens during specimen processing. Positive samples contained mixes of 10 different organisms detected by the FilmArray ME Panel with each targeted organism present at concentrations equivalent to approximately 3× LoD. The concentration of each potentially interfering substance added to each sample was equal to or greater than the highest level expected to be present in CSF. Contrived samples without potentially interfering substances added served as positive controls and a potentially interfering substance in negative sample matrix served as a negative or substance-only control. Samples containing each substance were evaluated for effects of the substance on the internal pouch control assays as well as effects on the ability of the FilmArray ME Panel to provide accurate organism test results compared to the control samples.

Interference was observed in the form of false negative results for *E. coli* and Enterovirus in samples containing high protein concentrations (albumin >15 mg/mL). This observed interference was further supported by overall higher Cp values for the yeast RNA process control (internal control). Additional testing was performed to evaluate samples with lower protein levels and results showed that interference was not observed at concentrations of 15mg/mL and lower.

Interference was also observed in the form of false negative results for several FilmArray ME analytes in samples containing bleach at a concentration > 0.1% (v/v).

All other substances evaluated did not interfere with FilmArray ME Panel results. The substances tested and study results are shown in the following table.

Results for Potentially Interfering Substances Tested on the FilmArray ME Panel

Endogenous Substances	Reference Concentration		Tested Concentration	Result
	Normal	Meningitis/Encephalitis		
Glucose	40-70 mg/dL (0.4-0.7 mg/mL)	≤ 70 mg/dL (≤ 0.7 mg/mL)	990 mg/dL (9.9 mg/mL)	No Interference
Lactate	10-20 mg/dL (0.1-0.2 mg/mL)	> 30 mg/dL (> 0.3 mg/mL)	220 mg/dL (2.2 mg/mL)	No Interference
Protein [Albumin]	Total Protein 45 mg/dL (0.45 mg/mL)	Total Protein 50-500 mg/dL (0.5-5.0 mg/mL)	5,000 mg/dL (50 mg/mL)	**Interference**
			4,000 mg/dL (40 mg/mL)	**Interference**
			1,500 mg/dL (15 mg/mL)	No Interference
			500 mg/dL (5 mg/mL)	No Interference
			100 mg/dL (1 mg/mL)	No Interference
Immunoglobulin (IgG)	0-8.0 mg/dL (0.0-0.08 mg/mL)	> 8.0 mg/dL (> 0.08 mg/mL)	1000 mg/dL (10 mg/mL)	No Interference
White Blood Cells (WBC)	0-20 cells/µL	5-5,000 cells/µL	10,000 cells/µL	No Interference
Human Genomic DNA	≤ 0.068 ng/µL	≤ 17 ng/µL	20 ng/µL	No Interference
Human Whole Blood	None		10% (v/v)	No Interference
Hemoglobin	None		200mg/dL (2 mg/mL)	No Interference
Transport Media**	**Concentration Tested**			**Result**
Trans-Isolate (T-I) Medium	50% (v/v)			No Interference
Viral Transport Medium (VTM)	50% (v/v)			No Interference
Disinfectants	**Concentration Tested**			**Result**
Ethanol	7% (v/v)			No Interference
Bleach	1% (v/v) [570 ppm chlorine in sample]			**Interference**
	0.1% (v/v) [57 ppm chlorine]			No Interference
	0.01% (v/v) [5.7 ppm chlorine]			No Interference

**CSF in transport media were not evaluated in the clinical studies and are not claimed for use with the FilmArray ME Panel.

i. Assay Cut-off:

The FilmArray ME Panel Melt Detector software determines whether a FilmArray ME Panel result is positive or negative using a predefined algorithm that includes Tm values, fluorescence values, and analysis of melting curves.

Initial melt ranges for each analyte-specific FilmArray ME Panel assay were determined based on a combination of mathematical modeling using known sequence variations of

different strains/isolates/variants of targeted organisms as well as data from testing of clinical specimens and known isolates.

After completion of the analytical and clinical studies on the FilmArray ME Panel, a final validation of the melt ranges was performed and included review of data from the Inclusivity study and clinical studies. The observed sensitivity and specificity rates for the individual melt curves and assay calls as compared to expert annotation was greater than 99.2% and 99.9% respectively. The sensitivity, specificity, and accuracy for the validation data was determined to be well above the acceptance criteria.

j. Specimen Stability

Stability of CSF specimens was evaluated to support labeling recommendations for storage of CSF samples at room temperature for up to 24 hours or at 2 - 8°C for up to seven days prior to testing. b(4) 3× LoD for each analyte, with the exception of *C. neoformans* and *C. gattii* which were evaluated at 15× and 30× LoD. Ten replicates were tested for each sample mix and storage condition. The analyses performed were both qualitative (percent agreement to the expected result) as well as numerical (change in Cp values). Study acceptance criteria required a minimum of 9/10 replicates detected.

Storage conditions were considered acceptable for use with the FilmArray ME Panel if accurate test results (equivalent to the non-stored samples (Day 0)) were obtained for at least nine of the ten replicates tested after storage.

As shown in the table below, study results demonstrated 10/10 positive results for all storage conditions for nine of ten analytes evaluated. For HSV-1, 9/10 replicates were positive, meeting the study acceptance criteria. Mean Cp values for all analytes were consistent between the control and stored samples, thereby further supporting the storage claims.

Additional testing was performed to evaluate room temperature storage for samples containing *C. neoformans* at an adjusted concentration of 300 CFU/mL (3× LoD). *C. neoformans* was detected as expected for 10/10 replicates.

The study data support the specimen handling recommendations provided in the FilmArray ME package insert.

Results for Specimen Stability Testing

FilmArray ME Panel Test Result	Isolate Tested	Concentration Tested (3x LoD)	# Detected/# Tested				
			No Storage (Control)	Ambient Storage	Refrigerated Storage		
				1 Day	Day 1	3 Days	7 Days
BACTERIA							
***Escherichia coli* K1**	type O18ac:K1:H7 b(4)	3.00E+03 CFU/mL	10/10	10/10	10/10	10/10	10/10
Haemophilus influenzae	type b, biotype I b(4)	3.00E+03 CFU/mL	10/10	10/10	10/10	10/10	10/10
Neisseria meningitidis	M-1574 [199/W135] b(4)	3.00E+03 CFU/mL	10/10	10/10	10/10	10/10	10/10
Streptococcus pneumoniae	SV 1/serotype 1 b(4)	300 cells/mL	10/10	10/10	10/10	10/10	10/10
VIRUSES							
Cytomegalovirus	AD-169 b(4)	300 $TCID_{50}$/mL	10/10	10/10	10/10	10/10	10/10
Enterovirus	Coxsackievirus A6, Gdula, group A b(4)	150 $TCID_{50}$/mL	10/10	10/10	10/10	10/10	10/10
Herpes simplex virus 1	MacIntyre b(4)	150 $TCID_{50}$/mL	10/10	9/10	10/10	10/10	10/10
Human parechovirus	type 3 b(4)	1.50E+03 $TCID_{50}$/mL	10/10	10/10	10/10	10/10	10/10
YEAST							
Cryptococcus neoformans/gattii	***C. neoformans***; H99, type strain b(4)	1.50E+03 CFU/mL	10/10	10/10	10/10	10/10	10/10
	C. gattii; A6MR38; AFLP6C; VGIIc b(4)	3.00E+03 CFU/mL	10/10	10/10	10/10	10/10	10/10

k. Fresh versus Frozen Study

An analytical study was performed to support the use of frozen specimens in the prospective, archived, and contrived specimen arms of the clinical study as well as in the reproducibility study. The study included a panel of 60 contrived specimens comprised of six representative FilmArray ME Panel analytes. Organisms evaluated included three bacteria know to be fastidious and/or known to demonstrate loss of viability upon freezing (*S. pneumoniae*, *N. meningitidis*, and *H. influenzae*), one yeast (*C. neoformans*), one DNA virus (HHV-6), and one RNA virus (HPeV).

Ten contrived samples were prepared for each analyte in pooled, residual negative CSF matrix. The majority of samples for each analyte were spiked at 2× LoD with the remaining samples having organism concentrations across the clinically relevant range (determined based on Cp values observed from previous FilmArray ME Panel positive test results). In some instances samples were included with concentrations below the

assay LoD. Each analyte was represented by six different strains with the exception of HHV6 for which two strains were evaluated. Following spiking, specimens were aliquoted and either tested fresh, or immediately frozen at <-70°C for at least 12 hours before testing.

Study results demonstrated 100% concordance for fresh and frozen samples for *H. influenzae*, *N. meningitidis*, and HPeV. *S. pneumoniae* demonstrated positive percent agreement (PPA) of 88.9% (one missed detection in a frozen specimen spiked at 2× LoD) and negative percent agreement (NPA) of 96% (one missed detection in a fresh specimen spiked at 0.2× LoD and one additional detection in an un-spiked frozen specimen). Two analytes demonstrated NPA of 98%: *C. neoformans/gattii* (one missed detection in a fresh specimen spiked at 2× LoD) and HHV-6 (one additional detection in an un-spiked frozen specimen). Analysis of overall Cp values between paired fresh and frozen samples demonstrated that differences in mean Cp values were within the expected system variability (typically within two cycles) and no significant trend toward higher or lower Cp values was observed between fresh and frozen samples.

An additional 22 clinical specimens that were tested fresh during the prospective clinical study were also re-tested after storage at -70°C. Retesting showed 100% detection after frozen storage. Similar to what was observed in the contrived specimens, the difference in average and median Cp values between fresh and frozen specimen testing was within the expected variability of the system.

The study results support inclusion of frozen specimens in the clinical and reproducibility studies.

2. Comparison Studies:

a. Clinical Comparison between FilmArray and FilmArray 2.0 systems:

To demonstrate that performance of the FilmArray ME Panel when used with the FilmArray 2.0 system is equivalent to FilmArray system, a combination of residual, de-identified CSF specimens and contrived CSF specimens covering all 14 analytes on the FilmArray ME Panel were evaluated. Specimens were identified as positive for FilmArray ME analytes at source laboratories or by culture (bacterial analytes) or PCR comparator methods (yeast and viruses) during the prospective FilmArray ME panel clinical study. The specimens were not chosen based on analyte levels but were chosen only for their previous analyte-specific positive test results and availability of sufficient volume for testing.

Contrived specimens were prepared as follows: For each analyte, leftover negative CSF specimens (negative as determined by FilmArray and comparator methods) were spiked with isolates of the organisms of interest at low concentrations (~3× LoD or below). Multiple strains for each organism were represented.

A total of 149 specimens were tested; 21 positive clinical specimens and128 contrived specimens. Positive contrived specimens were spiked with FilmArray ME Panel analytes at approximately 3× LoD or below. Specimens were split into two different aliquots for

testing with the FilmArray and the FilmArray 2.0 systems and all specimen identities were blinded to the operator.

Each analyte was represented a minimum of five times in the specimen set. All specimens were evaluated on both systems and three specimens with invalid runs (one on FilmArray and two on FilmArray 2.0) were excluded due to insufficient volume for retesting.

Test results were analyzed to compare performance between platforms. The FilmArray ME Panel demonstrated 100% concordance for 18 individual clinical specimens. Additionally, 100% concordance was also observed for nine out of 14 analytes for contrived specimens. The agreement for each analyte tested appears in the following table.

Clinical Performance Comparison Summary of FilmArray ME Panel on FilmArray 2.0 and FilmArray systems (Film Array 2.0 results are represented in the numerator and FilmArray results are represented in the denominator)

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		FA2.0/FA	%	95% CI	FA2.0/FA	%	95% CI
Bacteria							
E. coli K1	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	5/6	83.3%	43.7-97.0%	122/122	100%	97.0-100%
	Overall	5/6	83.3%	43.7-97.0%	143/143	100%	97.4-100%
H. influenzae	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	10/10	100%	72.3-100%	118/118	100%	96.9-100%
	Overall	10/10	100%	72.3-100%	139/139	100%	97.3-100%
L. monocytogenes	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	5/5	100%	56.6-100%	123/123	100%	97.0-100%
	Overall	5/5	100%	56.6-100%	144/144	100%	97.4-100%
N. meningitidis	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	11/11	100%	74.1-100%	117/117	100%	96.8-100%
	Overall	11/11	100%	74.1-100%	138/138	100%	97.3-100%
S. agalactiae	Clinical	1/1	100	20.7-100%	20/20	100%	83.9-100%
	Contrived	5/5	100%	56.6-100%	123/123	100%	97.0-100%
	Overall	6/6	100%	61.0-100%	143/143	100%	97.4-100%
S. pneumoniae	Clinical	1/1	100	20.7-100%	20/20	100%	83.9-100%
	Contrived	6/7	85.7%	48.7-97.4%	120/121	99.2%	95.5-99.9%
	Overall	7/8	87.5%	52.9-97.8%	140/141	99.3%	96.1-99.9%
Viruses							
CMV	Clinical	1/1	100	20.7-100%	20/20	100%	83.9-100%
	Contrived	5/5	100%	56.6-100%	123/123	100%	97.0-100%
	Overall	6/6	100%	61.0-100%	143/143	100%	97.4-100%
EV	Clinical	1/1	100	20.7-100%	20/20	100%	83.9-100%
	Contrived	11/12	91.7%	64.6-98.5%	114/116	98.3%	93.9-99.5%

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		FA2.0/FA	%	95% CI	FA2.0/FA	%	95% CI
	Overall	12/13	92.3%	66.7-98.6%	134/136	98.5%	94.8-99.6%
HSV-1	Clinical	3/3	100%	43.9-100%	18/18	100%	82.4-100%
	Contrived	3/3	100%	43.9-100%	125/125	100%	97.0-100%
	Overall	6/6	100%	61.0-100%	143/143	100%	97.4-100%
HSV-2	Clinical	2/2	100%	34.2-100%	19/19	100%	83.2-100%
	Contrived	3/3	100%	43.9-100%	125/125	100%	97.0-100%
	Overall	5/5	100%	56.6-100%	144/144	100%	97.4-100%
HHV-6	Clinical	3/3	100%	43.9-100%	18/18	100%	82.4-100%
	Contrived	9/9	100%	70.1-100%	117/119	98.3%	94.1-99.5%
	Overall	12/12	100%	75.8-100%	135/137	98.5%	94.8-99.6%
HPeV	Clinical	0/0	-	-	21/21	100%	84.5-100%
	Contrived	8/8	100%	67.6-100%	120/120	100%	96.9-100%
	Overall	8/8	100%	67.6-100%	141/141	100%	97.4-100%
VZV	Clinical	3/3	100	43.9-100	18/18	100%	82.4-100%
	Contrived	4/4	100%	51.0-100%	124/124	100%	97.0-100%
	Overall	7/7	100%	64.6-100%	142/142	100%	97.4-100%
Yeast							
C. neoformans/gattii	Clinical	2/2	100%	34.2-100%	19/19	100%	83.2-100%
	Contrived	15/15	100%	79.6-100%	112/113	99.1%	95.2-99.9%
	Overall	17/17	100%	81.6-100%	131/132	99.2%	95.8-99.9%
Overall Agreement		**117/120**	**97.5%**	**92.9-99.2%**	**1960/1966**	**99.7%**	**99.3-99.9%**

Overall PPA for clinical and contrived specimens combined was 97.5% with the lower bound of the two-sided 95% confidence interval (95% CI) at 92.9%, and overall NPA was 99.7% with the lower bound of the two-sided 95% CI at 99.3%.

An analysis of Tm values was conducted to compare the performance of the FilmArray ME Panel on both FilmArray and FilmArray 2.0 systems. Delta Tm values between the two systems were less than or equal to 0.4°C for all analytes, which passed the study acceptance criteria of less than 0.5°C.

Overall, the study results establish equivalent performance for clinical specimens when tested with the FilmArray ME Panel on the FilmArray and FilmArray 2.0 systems.

b. Matrix Equivalence Study

An equivalence study was performed to validate artificial CSF (aCSF) as a surrogate CSF sample matrix for contrived samples in analytical performance studies of the FilmArray ME Panel. The aCSF matrix mimics the composition of natural CSF and contains similar electrolyte concentrations. The equivalence study included paired samples comprised of four organism mixes prepared in natural and aCSF matrices. A total of 19 organisms covering all targeted FilmArray ME panel analytes were evaluated with samples containing mixtures of up to 5 different organisms. A minimum of four 10-fold dilutions

were tested per organism in each matrix, including concentrations below and above the LoD for each analyte. Four replicates were tested for each dilution. Where needed, testing of additional replicates or dilutions was performed in order to collect the necessary data to evaluate matrix effects for concentrations flanking the estimated LoD.

FilmArray ME panel results were evaluated for each analyte based on differences in qualitative detection as well as numerical analysis of Cp values for samples prepared in natural CSF and aCSF matrices. Study results showed no significant differences in qualitative detection for each organism evaluated (i.e., loss of detection occurred at similar dilutions for each matrix). In addition, analysis of mean Cp values showed no trend toward lower or higher values between samples prepared in natural or aCSF matrices.

Study results established that the FilmArray ME Panel provides equivalent results for contrived samples prepared in either aCSF matrix or a human clinical CSF matrix over a range of concentrations. Therefore aCSF was determined to be an acceptable sample matrix for preparation of samples for analytical studies.

3. Clinical Studies:

Prospective Clinical Study

The clinical performance of the FilmArray ME Panel was established during a multi-center study conducted at 11 geographically distinct U.S. study sites. A portion of specimens were collected and immediately frozen for later testing at the source laboratory. A total of 1643 prospective CSF specimens were acquired for the clinical study; 83 of these were excluded. The most common reasons for specimen exclusion were that specimens did not meet the inclusion criteria. Other reasons for exclusion included invalid daily external controls, FilmArray ME Panel run failure, testing with the incorrect FilmArray ME Panel pouch version, the specimen was beyond seven days of storage, lack of comparator test result, or internal control failure. The majority of CSF specimens were tested fresh; however a portion of prospective specimens were collected and immediately frozen for later testing. The final data set consisted of 1560 specimens, of which 545 (35%) had been previously frozen before testing. The following table provides a summary of demographic information for the 1560 specimens included in the prospective study.

Demographic Summary for Prospective Clinical Study

Prospective Study Specimens (%)	
Fresh	1015 (65%)
Frozen	545 (35%)
Total Specimens	1560
Sex	**Number of Specimens (%)**
Male	797 (51%)
Female	763 (49%)
Age Group	**Number of Specimens (%)**
< 2 mo.	299 (19%)
2-23 mo.	143 (9%)

2-17 years	197 (13%)
18-34 years	224 (14%)
35-64 years	522 (33%)
65+ years	175 (11%)
Status	**Number of Specimens (%)**
Outpatient	112 (7%)
Hospitalized	920 (59%)
Emergency	528 (34%)

The performance of the FilmArray ME Panel was evaluated by comparing the FilmArray ME Panel test result for each member of the panel with the appropriate comparator/reference methods shown in the following table.

Comparator Methods for FilmArray ME Panel Clinical Evaluation

FilmArray Analyte	Comparator Method	Comparator Test Location
E. coli K1	CSF bacterial culture	Source Laboratory
H. influenzae		
L. monocytogenes		
N. meningitidis		
S. agalactiae		
S. pneumoniae		
CMV	Two PCR assays with bi-directional sequencing[a]	BioFire Laboratory
EV		
HSV-1		
HSV-2		
HHV-6		
HPeV		
VZV		
C. neoformans/gattii		

[a]All assays targeted different nucleic acid sequences than those identified by the FilmArray ME Panel.

A total of 1560 specimens were evaluated in this study. Clinical sensitivity or positive percent agreement (PPA) was calculated as 100% x (TP / (TP + FN)). True positive (TP) indicates that both the FilmArray ME Panel and reference/comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the FilmArray ME Panel result was negative while the comparator result was positive. Specificity or negative percent agreement (NPA) was calculated as 100% x (TN / (TN + FP)). True negative (TN) indicates that both the FilmArray ME Panel and the reference/comparator method had negative results, and a false positive (FP) indicates that the FilmArray ME Panel result was positive but the comparator result was negative. The two-sided 95% confidence intervals were calculated.

Study results are summarized in the following table.

FilmArray ME Prospective Clinical Performance Summary[a]

Analyte		Sensitivity (compared to culture)			Specificity (compared to culture)		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Bacteria							
E. coli K1	Fresh	1/1	100	-	1014/1014	100	99.6-100
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	***2/2***	***100***	***34.2-100***	***1557/1558[b,c]***	***99.9***	***99.6-100***
H. influenzae	Fresh	1/1	100	-	1013/1014	99.9	99.4-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***1/1***	***100***	***-***	***1558/1559[d]***	***99.9***	***99.6-100***
L. monocytogenes	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***0/0***	***-***	***-***	***1560/1560***	***100***	***99.8-100***
N. meningitidis	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***0/0***	***-***	***-***	***1560/1560***	***100***	***99.8-100***
S. agalactiae	Fresh	0/1	0.0	-	1013/1014	99.9	99.4-100
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	***0/1[e]***	***0.0***	***-***	***1558/1559[e]***	***99.9***	***99.6-100***
S. pneumoniae	Fresh	2/2	100	34.2-100	1008/1013	99.5	98.8-99.8
	Frozen	2/2	100	34.2-100	536/543	98.7	97.4-99.4
	Overall	***4/4***	***100***	***51.0-100***	***1544/1556[f]***	***99.2***	***98.7-99.6***
Analyte		Positive Percent Agreement (compared to PCR with bi-directional sequencing)			Negative Percent Agreement (compared to PCR with bi-directional sequencing)		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Viruses							
CMV	Fresh	2/2	100	34.2-100	1010/1013	99.7	99.1-99.9
	Frozen	1/1	100	20.7-100	544/544	100	99.3-100
	Overall	***3/3***	***100***	***43.9-100***	***1554/1557[g]***	***99.8***	***99.4-99.9***
EV	Fresh	43/44	97.7	88.2-99.6	965/971	99.4	98.7-99.7
	Frozen	1/2	50.0	-	542/543	99.8	99.0-100
	Overall	***44/46[h]***	***95.7***	***85.5-98.8***	***1507/1514[h]***	***99.5***	***99.0-99.8***
HSV-1	Fresh	1/1	100	-	1013/1014	99.9	99.4-100
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	2/2	100	34.2-100	1556/1558[i]	99.9	99.5-100
HSV-2	Fresh	6/6	100	61.0-100	1008/1009	99.9	99.4-100
	Frozen	4/4	100	51.0-100	540/541	99.8	99.0-100
	Overall	10/10	100	72.2-100	1548/1550[j]	99.9	99.5-100
HHV-6	Fresh	13/15	86.7	62.1-96.3	997/1000	99.7	99.1-99.9
	Frozen	5/6	83.3	43.6-97.0	535/536	99.8	99.0-100

Analyte		Sensitivity (compared to culture)			Specificity (compared to culture)		
		TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
	Overall	18/21[k]	85.7	65.4-95.0	1532/1536[k]	99.7	99.3-99.9
HPeV	Fresh	9/9	100	70.1-100	1003/1006	99.7	99.1-99.9
	Frozen	0/0	-	-	545/545	100	99.3-100
	Overall	9/9	100	70.1-100	1548/1551[l]	99.8	99.4-99.9
VZV	Fresh	3/3	100	43.9-100	1010/1012	99.8	99.3-99.9
	Frozen	1/1	100	-	543/544	99.8	99.0-100
	Overall	4/4	100	51.0-100	1553/1556[m]	99.8	99.4-99.9
Yeast							
C. neoformans/gattii	Fresh	0/0	-	-	1015/1015	100	99.6-100
	Frozen	1/1	100	-	540/544	99.3	98.1-99.7
	Overall	***1/1***	***100***	***-***	***1555/1559[n]***	***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 Positive Percent Agreement and Negative Percent Agreement refer to all other analytes, for which PCR/sequencing assays were used as comparator methods.

[b] The FP specimen was negative for *E. coli* K1 when tested using an independent PCR assay targeting a nucleic acid region distinct from that identified by the FilmArray ME Panel. Meningitis was clinically excluded in this patient.

[c] An additional infant presented with CSF pleocytosis (WBC 3738) and *E. coli* bacteremia. CSF cultures and FilmArray ME Panel were negative, but no information regarding pre-treatment with antibiotics was available, and the patient was clinically diagnosed with meningitis.

[d] *H. influenzae* was detected in the single FP specimen using an independent PCR assay and was also observed via Gram stain; the subject from whom this specimen was collected received a physician diagnosis of gram-negative bacterial meningitis.

[e] The laboratory reported that *S. agalactiae* was present at a very low level (two colonies) for the FN specimen. The FP specimen was negative for *S. agalactiae* when tested using an independent PCR assay.

[f] *S. pneumoniae* was detected in 5/12 FP specimens using an independent PCR assay; additional information regarding seven unconfirmed FP specimens is detailed below in Table 10.

[g] CMV was detected in 1/3 FP specimens using an independent PCR assay.

[h] EV was detected in 2/2 FN specimens using an independent PCR assay; one specimen was positive upon FilmArray ME retest. EV was detected in 5/7 FP specimens using an independent PCR assay.

[i] Both FP specimens were negative for HSV-1 when tested using an independent PCR assay.

[j] HSV-2 was detected in 1/2 FP specimens using an independent PCR assay; the subject from whom this specimen was collected received a physician diagnosis of HSV meningitis.

[k] HHV-6 was detected in 2/3 FN and 1/4 FP specimens using an independent PCR assay.

[l] HPeV was detected in 1/3 FP specimens using an independent PCR assay; 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.

[m] VZV was detected in 1/3 FP specimens using an independent PCR assay; 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.

[n] *C. neoformans/gattii* was detected in 2/4 FP specimens using a commercially available antigen test. One FP specimen was positive by standard culture. Additional information regarding FilmArray ME Panel performance with respect to cryptococcal antigen testing is detailed below.

Of 12 specimens with false positive results for *S. pneumoniae*, seven could not be confirmed using an independent PCR assay. A review of de-identified subject medical data was conducted for the subjects from whom these specimens were collected and is summarized in the following table. None of the subjects had evidence of bacterial meningitis/encephalitis. The cause of these false positives was not determined.

Clinical Characteristics of Subjects with Unconfirmed False Positive *S. pneumoniae* Results

Subject age	CSF WBC	FilmArray Result	Comparator Culture/ Investigation PCR[a]	Diagnosis Reported in Medical Record
<2 mo	3	Pos	Neg/Neg	Infection, non-CNS (*S. agalactiae* urine culture)
65+	2	Pos	Neg/Neg	*Unable to obtain*
2-17	0	Pos	Neg/Neg	Infection, non-CNS (folliculitis)
<2 mo	3	Pos	Neg/Neg	Infection, non-CNS (Parainfluenza virus)
18-34	1	Pos	Neg/Neg	CNS disease, non-infectious (epilepsy)
35-64	1	Pos	Neg/Neg	Infection, non-CNS (Hep B), multiple myeloma
18-34	1	Pos	Neg/Neg	Infection, non-CNS (Bells' Palsy)

[a] Independent PCR assay is the same as described in footnote f in the table above.

The comparator method used to evaluate FilmArray ME Panel *C. neoformans/gattii* performance was PCR with bi-directional sequencing. FilmArray ME Panel performance for detection of *C. neoformans/gattii* was also calculated in comparison to specific testing for *Cryptococcus* that was performed by the laboratory based upon clinician test requests for a subset of subjects. For data that were available, FilmArray ME Panel performance is shown in the table below relative to cryptococcal antigen (CrAg) testing (N=196), standard culture (N=1560), and fungal culture (N=23). Notably, seven out of eight CrAg-positive specimens were discordant with FilmArray ME Panel results. All seven of these specimens were negative for *Cryptococcus* when tested with both PCR comparator assays. Medical chart review indicated that each subject was on antifungal therapy for treatment of cryptococcal meningitis or cryptococcosis at the time of specimen collection and/or had prior history of *Cryptococcus* infection. Therefore, positive antigen results for these patients in the absence of PCR and culture-based organism detection are likely due to antigen persistence rather than the presence of live organism.

FilmArray ME Panel *C. neoformans/gattii* assay performance relative to other comparator methods

Cryptococcus test comparator method	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
Cryptococcal Antigen	1/8[a]	12.5	2.2-47.1	187/188[b]	99.5	97.0-99.5
Standard Culture	2/3[c]	66.7	20.8-93.9	1554/1557[d]	99.8	99.4-99.9
Fungal Culture	0/0	-	-	22/23[e]	95.7	79.0-99.2

[a] Seven specimens were positive by CrAg testing performed at the clinical site, but were negative by standard of care culture, FilmArray ME Panel, and two comparator assays. All seven subjects from whom these specimens were collected received antifungal therapy prior to LP and/or had prior history of *Cryptococcus* infection. The eighth specimen was positive by CrAg, FilmArray ME Panel, and standard culture.

[b] *Cryptococcus* was detected in the single FP specimen using a CrAg testing kit at BioFire.

[c] The single FN specimen was also positive by standard culture, but negative by the FilmArray ME Panel and two comparator assays. The laboratory reported that only one colony was recovered.

[d] *Cryptococcus* was detected in 1/3 FP specimens using CrAg testing kits at BioFire (this is the same FP described in footnote a).

[e] The single FP specimen was negative by standard of care culture, CrAg testing performed at the clinical site, and negative by two comparator assays, but was positive by CrAg testing performed at BioFire (this is the same FP described in footnote a).

In the prospective clinical study, the FilmArray ME Panel reported a total of 5 specimens with multiple organism detections (0.3% of all specimens, 5/1560; and 3.7% of positive specimens, 5/136). Each multi-analyte detection contained two organisms, at least one of which was not detected by the reference/comparator method (i.e., each specimen contained at least one false positive result by the FilmArray ME Panel).The organism combinations detected by FilmArray ME Panel are shown in the following table.

Co-detection Combinations as Determined by the FilmArray ME Panel

Co-detection Combination	Number of Specimens	False Positive Analyte
CMV + *S. pneumoniae*	1	CMV
EV + HPeV	1	EV
HSV-1 + HHV-6	1	HSV-1
HSV-2 + *S. agalactiae*	1	S. agalactiae
S. pneumoniae + VZV	1	S. pneumoniae and VZV

The overall success rate for initial specimen tests in the prospective study was 98.9% (1560/1577); 17 tests were unsuccessful (11 due to incomplete tests and six due to control failures). Repeat testing was not possible to limited specimen volumes.

Testing of Preselected Archived Specimens

Several analytes were either not encountered or had a low prevalence in the prospective clinical study. To supplement the results of the prospective clinical study, an evaluation of 235 preselected archived specimens (of which 25 were negative) was performed. These specimens were archived clinical specimens that were selected because they had previously tested positive for one of the following analytes: *Cryptococcus*, CMV, *E. coli*, *H. influenzae*, HSV-1, HSV-2, HHV-6, HPeV, *L. monocytogenes*, *N. meningitidis*, *S. agalactiae*, *S. pneumoniae*, or VZV; or had been negative in previous laboratory testing. Prior to testing with the FilmArray ME Panel, the presence (or absence) of expected analytes was verified in each specimen using a confirmatory molecular test (e.g. PCR with bi-directional sequencing) Out of the 210 positives, the historical result was confirmed by the comparator method for 150 (150/210; 71.4%); only confirmed analytes were used in calculations of PPA, but all specimens were used for NPA analyses.

The specimens were organized into "test panels" and randomized such that the users performing the FilmArray ME Panel testing were blinded as to the expected test result. A summary of the available demographic information of the tested samples and the results of the FilmArray ME Panel testing are presented in the following tables.

Demographic Summary of Archived Specimens

Preselected Archived Specimens	
Total Specimens	235
Sex	**Number of Specimens (%)**
Male	70 (30%)
Female	90 (38%)
Unknown	75 (32%)
Age Group	**Number of Specimens (%)**
<2 mo	5 (2%)
2-23 mo	19 (8%)
2-17 yrs.	19 (8%)
18-34 yrs.	33 (14%)
35-64 yrs.	65 (28%)

65+ yrs.	26 (11%)
Unknown	68 (29%)

FilmArray ME Panel Archived Specimen Performance Data Summary

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
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 sequenced and identified as HHV-6A while 14 were HHV-6B. Of the four FilmArray ME Panel FN specimens, one was sequenced and identified as HHV-6A and the remaining three FN specimens were identified as HHV-6B. The resulting PPA was 50% (1/2); 95% CI 9.5 – 90.5% and 79% (11/14); 95% CI 52.4 – 92.4% for HHV-6A and HHV-6B, respectively.

[b] One specimen was sequenced and identified as *C. gattii* and 18 were *C. neoformans*.

Testing of Contrived Specimens

For some rare FilmArray ME Panel analytes, the prospective and archived clinical study arms included insufficient positive specimens to establish system performance. To supplement the prospective and archived data, an evaluation of contrived specimens was performed. Surrogate specimens were prepared using residual CSF specimens that had previously tested negative for all ME panel analytes by the FilmArray ME Panel and comparator methods. For each analyte, at least 25 specimens were spiked at 2× LoD and the remaining were spiked at four additional concentrations spanning the clinically relevant range using at least five different quantified strains for each organism. Specimens were prepared and randomized along with negative (unspiked) CSF specimens such that the analyte status of each contrived specimen was blinded to the users analyzing the specimens. Contrived specimens were frozen and then distributed to prospective clinical study sites for testing. The results of contrived specimens testing are presented in the following table.

FilmArray ME Panel Performance Using Contrived Specimens

Analyte	PPA			NPA		
	TP/(TP + FN)	%	95% CI	TN/(TN + FP)	%	95% CI
E. coli K1	47/49[a]	95.9	86.3-98.9	245/245	100	98.5-100
H. influenzae	50/50	100	92.9-100	243/244	99.5	97.7-99.9
L. monocytogenes	50/50	100	92.9-100	244/244	100	98.5-100
N. meningitidis	75/75	100	95.1-100	219/219	100	98.3-100
S. agalactiae	48/50[b]	96.0	86.5-98.9	244/244	100	98.5-100
CMV	47/49[c]	95.9	86.3-98.9	245/245	100	98.5-100
HHV-6	50/50	100	92.9-100	243/244	99.5	97.7-99.9
HPeV	50/50	100	92.9-100	244/244	100	98.5-100

[a] One *E coli* K1 false negative was observed at 2 × LoD and one *E coli* K1 false negative was observed at 0.2 × LoD.
[b] Both *S. agalactiae* false negatives were observed at 0.2 × LoD.
[c] Both CMV false negatives were observed at 0.2 × LoD.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The number and percentage of positive results as determined by the FilmArray ME Panel in the prospective clinical study are presented in the following table stratified by age group. Overall, the FilmArray ME Panel detected at least one organism or virus in a total of 136 prospective specimens (8.7% positivity rate) with a total of 141 analyte detections (co-detections were observed in five specimens).

Expected Values (as determined by the FilmArray ME Panel) Summary by Age Group for the Prospective Clinical Evaluation (February through September 2014)

FilmArray ME Panel Result	*Overall (n=1560)*	< 2 mo. (n=299)	2-23 mo. (n=143)	2-17 years (n=197)	18-34 years (n=224)	35-64 years (n=522)	65+ years (n=175)
Bacteria							
E. coli K1	*3 (0.2%)*	0 (0%)	1 (0.7%)	0 (0%)	0 (0%)	2 (0.4%)	0 (0%)
H. influenzae	*2 (0.1%)*	0 (0%)	1 (0.7%)	0 (0%)	0 (0%)	1 (0.2%)	0 (0%)
L. monocytogenes	*0 (0.0%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
N. meningitidis	*0 (0.0%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
S. agalactiae	*1 (0.1%)*	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.6%)
S. pneumoniae	*16 (1.0%)*	2 (0.7%)	2 (1.4%)	2 (1%)	3 (1.3%)	4 (0.8%)	3 (1.7%)
Viruses							
CMV	*6 (0.4%)*	4 (1.3%)	0 (0%)	1 (0.5%)	1 (0.4%)	0 (0%)	0 (0%)
EV	*51 (3.3%)*	31 (10.4%)	5 (3.5%)	11 (5.6%)	4 (1.8%)	0 (0%)	0 (0%)
HSV-1	*4 (0.3%)*	0 (0%)	2 (1.4%)	0 (0%)	0 (0%)	2 (0.4%)	0 (0%)
HSV-2	*12 (0.8%)*	0 (0%)	0 (0%)	0 (0%)	1 (0.4%)	8 (1.5%)	3 (1.7%)
HHV-6	*22 (1.4%)*	9 (3%)	7 (4.9%)	2 (1%)	3 (1.3%)	1 (0.2%)	0 (0%)

FilmArray ME Panel Result	*Overall (n=1560)*	< 2 mo. (n=299)	2-23 mo. (n=143)	2-17 years (n=197)	18-34 years (n=224)	35-64 years (n=522)	65+ years (n=175)
HPeV	*12 (0.8%)*	12 (4%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
VZV	*7 (0.4%)*	0 (0%)	0 (0%)	0 (0%)	3 (1.3%)	3 (0.6%)	1 (0.6%)
Yeast							
C. neoformans/gattii	*5 (0.3%)*	1 (0.3%)	0 (0%)	0 (0%)	1 (0.4%)	2 (0.4%)	1 (0.6%)

FilmArray ME Panel Positivity Rate In the Prospective Clinical Evaluation; Overall and By Age Group

Overall (n=1560)	
Negatives	1424 (91.3%)
Positives	136 (8.7%)
Single Detections	131 (8.4%)
Co-Detections	5 (0.3%)
Positivity by Age Group	
< 2 mo. (n=299)	58 (19.4%)
2-23 mo. (n=143)	17 (11.9%)
2-17 years (n=197)	15 (7.6%)
18-34 years (n=224)	15 (6.7%)
35-64 years (n=522)	23 (4.4%)
65+ years (n=175)	8 (4.6%)

M. Instrument Name

FilmArray and FilmArray 2.0

N. System Descriptions:

1. Modes of Operation:

   After samples and hydration reagent have been placed in the reagent pouch, the remaining processing steps are executed under control of the instrument.

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __X__ or No _____

3. Specimen Identification:

   Specimen identification can be entered manually or via barcode.

4. Specimen Sampling and Handling:

   The FilmArray ME Panel is intended for use with CSF specimens that have not been

centrifuged. The operator places a Hydration Injection Vial and a Sample Injection Vial into the FilmArray Pouch Loading Station. The operator hydrates the test pouch with the Hydration Injection Vial and then using a transfer pipette, adds~200 µl of CSF into the Sample Injection Vial. The operator removes the Sample Injection Vial containing the CSF from the Loading Station, inverts the vial at least three times to mix, and then inserts it into the Loading Station port where the proper amount of specimen is pulled into the FilmArray ME Panel pouch by vacuum. The FilmArray ME Panel is then placed onto the FilmArray instrument for testing.

5. Calibration:

   Not applicable

6. Quality Control:

   See Quality Control Section above (L.1.c "Traceability, Stability, Expected Values (controls, calibrators, or methods)")

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not Applicable

P. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809 and the specials controls for this device type.

Q. Identified Risks and Required Mitigations:

Identified Risks to Health	**Required Mitigations (See Section S below for Special Controls)**
Incorrect identification or lack of identification of a pathogenic microorganism by the device can lead to improper patient management	Special Controls (1), (2), (3), (4), and (5)
Failure to correctly interpret test results	Special Controls (6), (7), (8), and (9)
Failure to correctly operate the instrument	Special Control (10)

R. Benefit/Risk Analysis:

Summary	
Summary of the Benefit(s)	• The morbidity and mortality associated with some forms of meningitis and encephalitis can be substantial, therefore it is desirable to the patient to identify different causes of meningitis and/or encephalitis quickly. The primary benefit provided by the FilmArray ME Panel is the swift diagnosis of meningitis and/or encephalitis when compared to traditional culture. • Clinicians cannot distinguish between the various bacterial, viral and fungal causes of meningitis and/or encephalitis without additional laboratory testing. Also, cerebrospinal fluid must be obtained through an invasive procedure, and specimen volume may limit the number of tests that can be performed. The FilmArray ME Panel simultaneously tests for 14 analytes using only a small amount of CSF fluid, therefore additional fluid may be more readily available for additional testing. • Negative test results may provide additional clinician confidence when delaying empiric therapy or discontinuing empiric therapy. In these cases, patients may experience fewer side effects from antimicrobial therapy, and there may be additional benefits to antimicrobial stewardship programs. Such decisions will be at the discretion of the managing physician, who will have supplemental lab testing to guide the ultimate therapeutic decisions.
Summary of the Risk(s)	• False positive and false negative results, inaccurate identification of a pathogenic microorganism by the device, failure to correctly interpret test results, or failure to correctly operate the instrument are the primary risks associated with use of the FilmArray ME Panel. • A false positive result may lead to unnecessary antimicrobial therapy, with associated toxicities and side effects, including potential allergic reaction. These side effects from antimicrobial therapy are generally reversible, and limited to the duration of the antimicrobial agent. Certain viral analytes are known to reactivate in association with physiological stress or other infections. Positive results with the herpesviruses in particular may not reflect true causative infection, and could result in incorrect diagnosis. As a result, the true diagnosis could be delayed or missed, including non-infectious diseases or other infectious pathogens. The risk of a false-positive result is mitigated by device use in association with traditional culture as noted in the device's labeling. • False negative results could result in delayed diagnosis of meningitis and/or encephalitis, or delayed initiation of appropriate antimicrobial therapy. An incorrect identification could result in inappropriate antimicrobial therapy. As a result, the patient could experience a delay in effective antimicrobial therapy. The risk of a false-negative result is mitigated by device use in association with traditional culture as noted in the device's labeling.

Summary of Other Factors	None.
Conclusions Do the probable benefits outweigh the probable risks?	The probable benefits of the FilmArray ME Panel likely outweigh the potential risks in light of the listed special controls and applicable general controls, including design controls. The FilmArray ME Panel is the first of its kind, and represents a potential for patient benefit through more rapid diagnosis of meningitis and/or encephalitis. Potential risks associated with false positive or false negative results may be mitigated by use of traditional bacterial and fungal cultures, which would be necessary to recover the organism for further identification and susceptibility testing even without the FilmArray ME Panel. Data obtained from additional studies with contrived specimens demonstrated high sensitivity. The prospective clinical study found that the sensitivity for some analytes is low but given the small number of positive samples, it is difficult to establish with statistical significance how the FilmArray ME Panel will perform in real-world use. When used in conjunction with traditional cultures and clinical evaluation, much of the risk presented by this device can be mitigated. End user education will also allow clinicians to make informed judgements about how they will implement the FilmArray ME Panel in their clinical practice. Ultimately, the majority of risks associated with the FilmArray ME Panel may be minimized with appropriate precautions and the FilmArray ME Panel may provide substantial benefit to patients.

S. Conclusion:

The information provided in this *de novo* submission is sufficient to classify this device into class II under regulation 21 CFR 866.3970. FDA believes that the stated special controls, and applicable general controls, including design controls, provide reasonable assurance of the safety and effectiveness of the device type. The device is classified under the following:

Product Code: PLO, OOI, NSU

Device Type: Device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid.

Class: II (special controls)

Regulation: 21 CFR 866.3970

(a) Identification. A device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid is identified as a qualitative *in vitro* device intended for the detection and identification of microbial-associated nucleic acid sequences from patients suspected of meningitis or encephalitis. A device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid is intended to aid in the diagnosis of meningitis or

encephalitis when used in conjunction with clinical signs and symptoms and other clinical and laboratory findings.

(b) Classification. Class II (special controls). A device to detect and identify microbial pathogen nucleic acids in cerebrospinal fluid must comply with the following special controls:

1) Premarket notification submissions must include detailed device description documentation, including the device components, ancillary reagents required but not provided, and a detailed explanation of the methodology including primer/probe sequence, design, and rationale for sequence selection.
2) Premarket notification submissions must include detailed documentation from the following analytical studies: Analytical sensitivity (Limit of Detection), inclusivity, reproducibility, interference, cross reactivity, and specimen stability.
3) Premarket notification submissions must include detailed documentation from a clinical study. The study, performed on a study population consistent with the intended use population, must compare the device performance to results obtained from well-accepted comparator methods.
4) Premarket notification submissions must include detailed documentation for device software, including, but not limited to, software applications and hardware-based devices that incorporate software.
5) The Intended Use statement in the device labeling must include a statement that the device is intended to be used in conjunction with standard of care culture.
6) A detailed explanation of the interpretation of results and acceptance criteria must be included in the device's 21 CFR 809.10(b)(9) compliant labeling.
7) The device labeling must include a limitation that negative results do not preclude the possibility of central nervous system infection.
8) The device labeling must include a limitation that device results are not intended to be used as the sole basis for diagnosis, treatment, or other patient management decisions.
9) The device labeling must include a limitation stating that positive results do not mean that the organism detected is infectious or is the causative agent for clinical symptoms.
10) As part of the risk management activities performed as part of your 21 CFR 820.30 design controls, you must document an appropriate end user device training program that will be offered as part of your efforts to mitigate the risk of failure to correctly operate the instrument.