FilmArray
Meningitis/Encephalitis Panel

Christine C. Ginocchio, PhD, MT (ASCP)
VP, Scientific and Medical Affairs, BioFire Diagnostics
VP, Global Microbiology, bioMerieux
Professor of Medicine, Hofstra North Shore-LIJ School of Medicine, NY
Delayed Treatment: Increased Morbidity and Mortality

- The causative agents of meningitis or encephalitis should be rapidly identified to guide appropriate patient management, but are many times impossible to identify based on clinical indications alone due to overlapping symptoms\(^1,2\)
- Delays in appropriate therapy can be associated with adverse outcomes\(^1\)

**Bacterial**
- Permanent brain and nerve damage
- Behavioral changes
- Cognitive disabilities
- Lack of muscle control
- Seizures

**Viral**
- Brain damage, including behavioral and personality changes and memory and speech problems
- Focal neurological signs
- Seizures

**Fungal**
- Increased intracranial pressure
- Hydrocephalus
- IRIS
- Blindness, sometimes with optic atrophy

Early diagnosis facilitates timely and appropriate therapeutic interventions and can minimize the risks of adverse outcomes and mortality\(^2\)
Traditional Diagnostics

**CSF Examination**
- Cell count
- Protein
- Glucose

**CSF Culture**
- Pathogen-specific media
- Bacterial culture
- Viral culture
- Fungal culture

**Gram Stain**
- Crystal violet
- Iodine
- Alcohol
- Safranin

**India Ink Stain**

**Rapid Latex Agglutination Test**
- Positive
- Negative

**Traditional PCR**

<table>
<thead>
<tr>
<th>Test</th>
<th>Time Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF Examination</td>
<td>&lt;1 hr</td>
</tr>
<tr>
<td>CSF Culture</td>
<td>72 hr-14 d</td>
</tr>
<tr>
<td>Gram Stain</td>
<td>&lt;1 hr</td>
</tr>
<tr>
<td>India Ink Stain</td>
<td>&lt;1 hr</td>
</tr>
<tr>
<td>Rapid Latex Agglutination Test</td>
<td>2-4 hr</td>
</tr>
<tr>
<td>Traditional PCR</td>
<td>1-24 hr</td>
</tr>
</tbody>
</table>

CSF = cerebrospinal fluid; PCR = polymerase chain reaction.

Challenges in Diagnosing Meningitis and Encephalitis Infections

- Meningitis and encephalitis often present with similar symptoms, sometimes as flu-like symptoms\(^1\)
- The causative agents underlying the disease may not be distinguishable based on clinical symptoms alone\(^2\)

**Challenges associated with currently available testing methods\(^1,3\):**

- Time-consuming
- Technically complex/requires specific expertise
- Lack sensitivity and specificity
- Accuracy may be affected by antibiotic administration
- Small volume of CSF obtained
- Physician must choose which tests to select based on symptoms and available CSF volume
- Need to order multiple tests specific for suspected organisms

CSF = cerebrospinal fluid.

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

**Viruses**
*Cytomegalovirus (CMV)*
*Enterovirus*
*Herpes simplex virus 1 (HSV-1)*
*Herpes simplex virus 2 (HSV-2)*
*Human herpesvirus 6 (HHV-6)*
*Human parechovirus*
*Varicella zoster virus (VZV)*

**Fungi**
*Cryptococcus neoformans/gattii*
Comprehensive Diagnostics for the Most Common Pathogens

- *E. coli*
- GBS
- *L. monocytogenes*
- *H. influenzae*
- HSV-1, 2
- EV, PeV

- *S. pneumoniae*
- *N. meningitidis*
- *H. influenzae* (NT)
- EV, PeV
- HHV-6
- EBV, CMV
- Arboviruses

- *N. meningitidis*
- *S. pneumoniae*
- *H. influenzae* (NT)
- EV, PeV
- HSV-1, 2
- EBV, CMV
- Arboviruses

- *S. pneumoniae*
- *N. meningitidis*
- *H. influenzae* (NT)
- Gram neg (*K. kingae*)
- HSV-1, 2, VZV (zoster)
- EV
- Arboviruses
- Neuroborelliosis

- **All above +**
- *L. monocytogenes*
- CMV, EBV
- HIV
- HHV-6
- Polyomaviruses (BK)
- Aspergillosis
- Nocardiosis
- Cryptococcosis
- *Toxoplasmosis*
- Neurosyphilis
- Gram negatives
- Gram positives
- ++++++++
BioFire FilmArray® System

Setting up the FilmArray® is Easy – Sample in, Results out

- Insert Pouch into Loading Station
- Inject Hydration Solution
- Inject Sample
- Add Pouch to FilmArray and Start Run
BioFire FilmArray® Pouch

Sample extraction and purification
1st stage multiplex PCR
2nd stage PCR
1. Sample moves into lysis chamber. Cells and pathogens are lysed by bead beating, releasing nucleic acids. Fluorescent double-stranded DNA binding dye monitors each reaction.
Automated Results Analysis

- 102 individual 2\textsuperscript{nd} stage PCR wells
- Each well contains one reaction
- 2/3 wells must be positive
- Melt curves generated for each well: must be within specific ranges

Process Control

- Freeze-dried \textit{Schizosaccharomyces pombe} organism
- Re-suspended with specimen
- \textbf{PCR II Control}
  - DNA template spotted on the array
Intended Use

- **Qualitative** test for bacteria, viruses and yeast
- **CSF** (200 μl) obtained via **lumbar puncture** from individuals with signs and/or symptoms of meningitis and/or encephalitis.
- Not centrifuged, xanthrochromic and bloody acceptable
- Not intended for testing of specimens collected from indwelling CNS medical devices (shunts)
- “Aid in diagnosis” – must be used with other clinical, lab and epidemiological data
- **Culture is necessary** for organism recovery, typing, and antimicrobial susceptibility testing of bacterial agents

**FilmArray and FilmArray 2.0 Systems**
Performance Studies

- Analytical Validation
  - Limit of Detection (LoD)
  - Reactivity
  - Precision/Reproducibility

- Clinical Validation
  - Prospective clinical trial
  - Archived specimen testing
  - Contrived specimen testing
ANALYTICAL STUDIES
## Limit of Detection (LoD) 95% Detection Level

LoD is 100 – 1,000 CFU/mL or cells/mL for all bacteria & yeast

<table>
<thead>
<tr>
<th>ME Panel Test Result</th>
<th>Species/Isolate Tested</th>
<th>LoD Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BACTERIA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> K1</td>
<td><em>E. coli</em> K1, O18ac:K1:H7 ATCC 700973</td>
<td>$1 \times 10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td><em>H. influenzae</em>, type b, biotype I ATCC 10211</td>
<td>$1 \times 10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td><em>L. monocytogenes</em>, type 4b ATCC 13932</td>
<td>$1 \times 10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td><em>N. meningitidis</em>, type W135 ATCC 43744</td>
<td>100 CFU/mL</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td><em>S. agalactiae</em>, group B ATCC 13813</td>
<td>$1 \times 10^3$ CFU/mL</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td><em>S. pneumoniae</em>, serotype 1</td>
<td>100 cells/mL*</td>
</tr>
<tr>
<td><strong>YEAST</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. neoformans/gattii</em></td>
<td><em>C. neoformans</em> ATCC 20882</td>
<td>100 CFU/mL</td>
</tr>
<tr>
<td></td>
<td><em>C. gattii</em> ATCC MYA-4877</td>
<td>100 CFU/mL</td>
</tr>
</tbody>
</table>

*cells/mL is based on OD$_{600}$ reading – no CFU/mL determined
# Limit of Detection (LoD) 95% Detection Level

LoD is 5-500 TCID\textsubscript{50}/mL or 100 – 1,000 copies/mL for all viruses

<table>
<thead>
<tr>
<th>ME Panel Test Result</th>
<th>Species/Isolate Tested</th>
<th>LoD Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>viruses</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>CMV, strain AD-169&lt;br&gt;Zeptometrix 0810003CF</td>
<td>100 TCID\textsubscript{50}/mL (4.30 × 10³ copies/mL)</td>
</tr>
<tr>
<td>EV</td>
<td>Coxsackievirus A6, species A, strain Gdula&lt;br&gt;ATCC VR-1801</td>
<td>50 TCID\textsubscript{50}/mL</td>
</tr>
<tr>
<td></td>
<td>Coxsackievirus A9, species B&lt;br&gt;Zeptometrix 0810017CF</td>
<td>5 TCID\textsubscript{50}/mL</td>
</tr>
<tr>
<td></td>
<td>Coxsackievirus A17, species C, strain G-12&lt;br&gt;ATCC VR-1023</td>
<td>5 TCID\textsubscript{50}/mL</td>
</tr>
<tr>
<td></td>
<td>EV 70, species D, strain J670/71&lt;br&gt;ATCC VR-836</td>
<td>50 TCID\textsubscript{50}/mL</td>
</tr>
<tr>
<td>HSV-1</td>
<td>HSV-1, strain MacIntyre&lt;br&gt;Zeptometrix 0810005CF</td>
<td>250 TCID\textsubscript{50}/mL (1.51 × 10³ copies/mL)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>HSV-2, strain MS&lt;br&gt;Zeptometrix 0810006CF</td>
<td>50 TCID\textsubscript{50}/mL (1.29 × 10³ copies/mL)</td>
</tr>
<tr>
<td>HHV-6</td>
<td>HHV-6A, strain U1102, NCPV 0003121v&lt;br&gt;HHV-6B, strain HST, NCPV 0006111v</td>
<td>1 × 10⁴ copies/mL</td>
</tr>
<tr>
<td>HPeV</td>
<td>HPeV, type 3&lt;br&gt;Zeptometrix 0810147CF</td>
<td>500 TCID\textsubscript{50}/mL</td>
</tr>
<tr>
<td>VZV</td>
<td>VZV, strain Ellen&lt;br&gt;Zeptometrix 0810171CF</td>
<td>0.10 TCID\textsubscript{50}/mL (1.66 × 10³ copies/mL)</td>
</tr>
</tbody>
</table>
## Summary of ME Analytical Reactivity (Inclusivity)

<table>
<thead>
<tr>
<th>FilmArray ME Panel Test Result</th>
<th># Tested and Detected</th>
<th>Concentration Detected</th>
<th>Isolates Tested and Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> K1</td>
<td>5</td>
<td>≤ 3,000 CFU/mL</td>
<td><em>E. coli</em> strains of the K1 serotype only</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>9</td>
<td>≤ 3,000 CFU/mL</td>
<td>Non-typeable and typeable (types a-f) strains of <em>H. influenzae</em></td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>6</td>
<td>≤ 3,000 CFU/mL</td>
<td>Types 1/2a, 1/2b, and 4b of <em>L. monocytogenes</em>&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>7</td>
<td>≤ 300 CFU/mL</td>
<td>Encapsulated <em>N. meningitidis</em> (serotypes W135, A, B, C, D, Y and DNA from a strains with a variant <em>ctrA</em> gene)</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>5</td>
<td>≤ 3,000 CFU/mL</td>
<td>Multiple serotypes or isolates of <em>S. agalactiae</em> (Group B <em>Streptococcus</em>)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>6</td>
<td>≤ 300 cells/mL</td>
<td>Multiple serotypes of <em>S. pneumoniae</em></td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>5</td>
<td>≤ 300 TCID&lt;sub&gt;50&lt;/sub&gt;/mL (≤ 1 × 10&lt;sup&gt;4&lt;/sup&gt; copies/mL)</td>
<td>Multiple strains of Cytomegalovirus (CMV).</td>
</tr>
<tr>
<td>EV</td>
<td>18</td>
<td>≤ 50 TCID&lt;sub&gt;50&lt;/sub&gt;/mL</td>
<td>Representative isolates from all species (A-D) and several serotypes of human Enterovirus, Coxsackievirus, and Echovirus&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSV-1</td>
<td>5</td>
<td>≤ 750 TCID&lt;sub&gt;50&lt;/sub&gt;/mL (≤ 4.5 × 10&lt;sup&gt;3&lt;/sup&gt; copies/mL)</td>
<td>Multiple strains of Herpes simplex virus 1 (HSV-1)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>5</td>
<td>≤ 150 TCID&lt;sub&gt;50&lt;/sub&gt;/mL (≤ 4 × 10&lt;sup&gt;3&lt;/sup&gt; copies/mL)</td>
<td>Multiple strains of Herpes simplex virus 2 (HSV-2)</td>
</tr>
<tr>
<td>HHV-6</td>
<td>4</td>
<td>≤ 3 × 10&lt;sup&gt;4&lt;/sup&gt; copies/mL</td>
<td>A and B variants of Human herpesvirus 6 (HHV-6)</td>
</tr>
<tr>
<td>HPeV</td>
<td>6</td>
<td>500 - 5,000 TCID&lt;sub&gt;50&lt;/sub&gt;/mL</td>
<td>Serotypes 1-6 of Human parechovirus (HPeV)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>VZV</td>
<td>5</td>
<td>≤ 0.3 TCID&lt;sub&gt;50&lt;/sub&gt;/mL (≤ 5 × 10&lt;sup&gt;3&lt;/sup&gt; copies/mL)</td>
<td>Multiple strains of Varicella zoster virus (VZV)</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. neoformans/gattii</em></td>
<td>10</td>
<td>≤ 300 CFU/mL</td>
<td>Multiple strains, serotypes, and genotypes of <em>Cryptococcus neoformans</em> and <em>Cryptococcus gattii</em></td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes reference to a variant gene.  
<sup>b</sup> Includes reference to several serotypes within species A-D.  
<sup>c</sup> Includes reference to serotypes 1-6.
Analytical Specificity (Exclusivity/Cross-Reactivity)

>100 off-panel isolates tested at high concentration

Minor cross-reactivity identified:

- Enterovirus (EV2) assay will cross-react with various rhinoviruses
- *H. influenzae* assay (*H. influenzae* 1) will cross-react with *H. haemolyticus*
- *C. neoformans/gattii* assay will cross-react with *C. amylolentus*

Rhinovirus and *H. haemolyticus* are rarely isolated from CSF – but can be in respiratory tract – avoid sample contamination

*C. amylolentus is not isolated from humans*
Precision/Reproducibility – Study Design

- Every analyte tested @ 3 levels
  - Moderate Positive (3× LoD)
    - Expect positive results in >95% of samples
  - Low Positive (1× LoD)
    - Expect positive results in ≥95% of samples
  - Negative
    - Expect negative results in all samples

- Testing occurs:
  - At 3 test sites
  - Multiple runs per day, on multiple (5) days
  - On multiple instruments/ 2.0 systems
  - By multiple operators (2 per day per site)
  - With multiple pouch/reagent lots
## Precision/Reproducibility Results

Expected qualitative results (Detected/Not Detected) reported at all sites, with all instruments/systems, etc.

<table>
<thead>
<tr>
<th>Test Result (Organism/Isolate Tested)</th>
<th>Concentration Tested</th>
<th>Expected Result</th>
<th>Agreement with Expected Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>FilmArray</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Site A</td>
</tr>
<tr>
<td><strong>BACTERIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> K1 (ATCC 700973)</td>
<td>Moderate Positive 3 x LoD 3 x 10³ CFU/mL</td>
<td>Detection</td>
<td>30/30</td>
</tr>
<tr>
<td></td>
<td>Low Positive 1 x LoD 1 x 10³ CFU/mL</td>
<td>Detection</td>
<td>30/30</td>
</tr>
<tr>
<td></td>
<td>Negative (No analyte)</td>
<td>Not Detected</td>
<td>60/60</td>
</tr>
</tbody>
</table>
CLINICAL PERFORMANCE
PROSPECTIVE, ARCHIVED, CONTRIVED TESTING
Prospective Study

Overview

- In hospital laboratories
- Prospectively-collected patient specimens
- Residual specimens leftover from clinician order for CSF bacterial culture
- At least 1500 tests to show performance in intended use setting
Comparator Methods

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

PCR & Sequencing*
- *Cytomegalovirus*
- *Epstein-Barr virus*
- *Herpes simplex virus 1*
- *Herpes simplex virus 2*
- *Human herpesvirus 6*
- *Varciella zoster virus*
- *Human parechovirus*
- *Human enterovirus*

*Cryptococcus neoformans/gattii*

2 PCR assays + sequencing
Data Collection

**Basic Demographics**
- Age range (<2 mo, 2 – 23 mo, 2 – 17 yrs, 18 – 34 yrs, 35 – 64 yrs, >65 yrs)
- Sex
- Status (ED, inpatient, outpatient)

**Results of bacterial culture**

**Results of any other CSF testing ordered**

**CSF chemistry (protein, glucose, WBC + differential)**

**For positives:**
- Abx issued within 24 hrs prior to LP
- Immune status
- Clinician final diagnosis
Enrollment Summary

- 1560 prospectively-collected, residual specimens
  - Of which 545 (35%) had been previously frozen*

- Feb – Sept 2014
- 11 U.S. Study Sites
- All age groups included
  - (41% under 18)

- Mostly hospitalized (59%) or ED (34%)

*IUO pouches available June 2014
98.9% of specimens were successfully tested on the initial test

- 11 instrument / software errors (~0.7%)
- 6 pouch control failures (~0.4%)
Overall Positivity = 8.7%

Positivity by Age Group:
- < 2 mo. (n=299): 19.40%
- 2-23 mo. (n=143): 11.90%
- 2-17 years (n=197): 7.60%
- 18-34 years (n=224): 6.70%
- 35-64 years (n=522): 4.40%
- 65+ years (n=175): 4.60%

Overall:
- Viruses: 81%
- Bacteria: 16%
- Yeast: 3%
## Prevalence by FilmArray

**February to September 2014 - United States**

<table>
<thead>
<tr>
<th>FilmArray ME Panel Result</th>
<th>Overall (n=1560)</th>
<th>&lt; 2 mo. (n=299)</th>
<th>2-23 mo. (n=143)</th>
<th>2-17 years (n=197)</th>
<th>18-34 years (n=224)</th>
<th>35-64 years (n=522)</th>
<th>65+ years (n=175)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli K1</em></td>
<td>3 (0.2%)</td>
<td>0 (0%)</td>
<td>1 (0.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (0.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>2 (0.1%)</td>
<td>0 (0%)</td>
<td>1 (0.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>0 (0.0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>0 (0.0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>1 (0.1%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>16 (1.0%)</td>
<td>2 (0.7%)</td>
<td>2 (1.4%)</td>
<td>2 (1%)</td>
<td>3 (1.3%)</td>
<td>4 (0.8%)</td>
<td>3 (1.7%)</td>
</tr>
<tr>
<td>Viruses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>6 (0.4%)</td>
<td>4 (1.3%)</td>
<td>0 (0%)</td>
<td>1 (0.5%)</td>
<td>1 (0.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>EV</td>
<td>51 (3.3%)</td>
<td>31 (10.4%)</td>
<td>5 (3.5%)</td>
<td>11 (5.6%)</td>
<td>4 (1.8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>HSV-1</td>
<td>4 (0.3%)</td>
<td>0 (0%)</td>
<td>2 (1.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>2 (0.4%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>HSV-2</td>
<td>12 (0.8%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.4%)</td>
<td>8 (1.5%)</td>
<td>3 (1.7%)</td>
</tr>
<tr>
<td>HHV-6</td>
<td>22 (1.4%)</td>
<td>9 (3%)</td>
<td>7 (4.9%)</td>
<td>2 (1%)</td>
<td>3 (1.3%)</td>
<td>1 (0.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>HPeV</td>
<td>12 (0.8%)</td>
<td>12 (4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VZV</td>
<td>7 (0.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>3 (1.3%)</td>
<td>3 (0.6%)</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Yeast</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. neoformans/gattii</em></td>
<td>5 (0.3%)</td>
<td>1 (0.3%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.4%)</td>
<td>2 (0.4%)</td>
<td>1 (0.6%)</td>
</tr>
</tbody>
</table>
## Summary of Prospective Data

### Bacteria

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP/(TP + FN)</td>
<td>%</td>
</tr>
<tr>
<td><strong>E. coli K1</strong></td>
<td>2/2</td>
<td>100</td>
</tr>
<tr>
<td><strong>H. influenzae</strong></td>
<td>1/1</td>
<td>100</td>
</tr>
<tr>
<td><strong>L. monocytogenes</strong></td>
<td>0/0</td>
<td>-</td>
</tr>
<tr>
<td><strong>N. meningitidis</strong></td>
<td>0/0</td>
<td>-</td>
</tr>
<tr>
<td><strong>S. agalactiae</strong></td>
<td>0/1</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>S. pneumoniae</strong></td>
<td>4/4</td>
<td>100</td>
</tr>
</tbody>
</table>

### Viruses

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Positive Percent Agreement</th>
<th>Negative Percent Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP/(TP + FN)</td>
<td>%</td>
</tr>
<tr>
<td><strong>CMV</strong></td>
<td>3/3</td>
<td>100</td>
</tr>
<tr>
<td><strong>EV</strong></td>
<td>44/46</td>
<td>95.7</td>
</tr>
<tr>
<td><strong>HSV-1</strong></td>
<td>2/2</td>
<td>100</td>
</tr>
<tr>
<td><strong>HSV-2</strong></td>
<td>10/10</td>
<td>100</td>
</tr>
<tr>
<td><strong>HHV-6</strong></td>
<td>18/21</td>
<td>85.7</td>
</tr>
<tr>
<td><strong>HPeV</strong></td>
<td>9/9</td>
<td>100</td>
</tr>
<tr>
<td><strong>VZV</strong></td>
<td>4/4</td>
<td>100</td>
</tr>
</tbody>
</table>

### Yeast

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP/(TP + FN)</td>
<td>%</td>
</tr>
<tr>
<td><strong>C. neoformans/gattii</strong></td>
<td>1/1</td>
<td>100</td>
</tr>
</tbody>
</table>
Concern for False Positives

Laboratory Precautions
- Due to the sensitive nature of the FilmArray ME Panel, it is important to guard against contamination.

Limitations in Product Literature
- *S. pneumoniae* and *H. influenzae* can be shed from the respiratory tract of healthy individuals.
- HSV-1 may also be shed from individuals with active or recurrent cold sores.
- Particular attention should be given to the Laboratory Precautions noted under the Warnings and Precautions section.
- Caution should also be exercised during specimen collection and testing to prevent contamination leading to false positive results.
- Results from this test *must be correlated with the clinical history, epidemiological data, and other data available* to the clinician evaluating the patient.
PCR negative/CrAg positive

- Low level CrAg titers may persist for extended periods of time following appropriate therapy and the resolution of infection
  - May be indicative of cryptococcal immune reconstitution inflammatory syndrome (IRIS) with history of cryptococcal meningitis and sterile cultures

- Medical chart review indicated that each subject with positive CrAg and negative FilmArray 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.
  - Likely due to antigen persistence rather than the presence of live organism
Co-Detections

136 Positive Specimens
141 Positive Results
5 dual detections

Recommend retesting specimens showing multiple detections, to rule out potential contamination

<table>
<thead>
<tr>
<th>Distinct Co-Detection Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte 1</td>
</tr>
<tr>
<td>CMV</td>
</tr>
<tr>
<td>EV</td>
</tr>
<tr>
<td>HSV-1</td>
</tr>
<tr>
<td>HSV-2</td>
</tr>
<tr>
<td>VZV</td>
</tr>
</tbody>
</table>
### Considerations for Herpesvirus Detections

- HHV-6 or CMV can exist in latent form that is reactivated during infection due to other pathogens, including agents not detected by the FilmArray ME panel that may cause meningitis/encephalitis (e.g., *Mycobacterium tuberculosis* or HIV).

- When detected by the FilmArray ME, HHV-6 or CMV should be considered as the likely cause of meningitis/encephalitis only in appropriate clinical settings and following expert consultation.

<table>
<thead>
<tr>
<th>Run Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample ID:</strong></td>
</tr>
<tr>
<td><strong>Detected:</strong></td>
</tr>
<tr>
<td><strong>Run Date:</strong></td>
</tr>
<tr>
<td><strong>Controls:</strong></td>
</tr>
</tbody>
</table>

**WARNING:** The FilmArray ME Panel does not distinguish between latent and active CMV and HHV-6 infections. Detection of these viruses may indicate primary infection, secondary reactivation, or the presence of latent virus. Results should always be interpreted in conjunction with other clinical, laboratory, and epidemiological information.

- Viral shedding into the CSF often occurs in cases of zoster (shingles; caused by reactivation of VZV). VZV may not be the cause of CNS disease in some cases.
Archived Study

**Purpose:**
- Supplement prospective study by testing known positive specimens

**Workflow:**
- Lab ID is confirmed by molecular assay

**Data Analysis:**
- Only confirmed specimens are considered for performance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Received</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em> K1</td>
<td>7</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>2</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>21</td>
</tr>
<tr>
<td>CMV</td>
<td>11</td>
</tr>
<tr>
<td>HSV-1</td>
<td>17</td>
</tr>
<tr>
<td>HSV-2</td>
<td>26</td>
</tr>
<tr>
<td>ARUP “HSV positive”</td>
<td>35</td>
</tr>
<tr>
<td>HHV-6</td>
<td>22</td>
</tr>
<tr>
<td>HPeV</td>
<td>6</td>
</tr>
<tr>
<td>VZV</td>
<td>29</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans/gattii</em></td>
<td>22</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>210</strong></td>
</tr>
</tbody>
</table>

Previous Panel Archived Confirmation:
RP 86% confirmed and GI 85% confirmed
# Archived Performance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Positive Percent Agreement</th>
<th></th>
<th>Negative Percent Agreement</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP/(TP + FN)</td>
<td>%</td>
<td>95% CI</td>
<td>TN/(TN + FP)</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em> K1</td>
<td>2/2</td>
<td>100</td>
<td>34.2-100</td>
<td>35/35</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>3/3</td>
<td>100</td>
<td>43.9-100</td>
<td>39/39</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>1/1</td>
<td>100</td>
<td>-</td>
<td>41/41</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>7/7</td>
<td>100</td>
<td>64.6-100</td>
<td>34/34</td>
</tr>
<tr>
<td><em>S. agalactiae</em></td>
<td>2/2</td>
<td>100</td>
<td>34.2-100</td>
<td>40/40</td>
</tr>
<tr>
<td><em>S. pneumoniae</em></td>
<td>17/17</td>
<td>100</td>
<td>81.6-100</td>
<td>21/21</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV</td>
<td>7/8</td>
<td>87.5</td>
<td>52.9-97.8</td>
<td>181/181</td>
</tr>
<tr>
<td>HSV-1</td>
<td>16/16</td>
<td>100</td>
<td>80.6-100</td>
<td>156/157</td>
</tr>
<tr>
<td>HSV-2</td>
<td>33/34</td>
<td>97.1</td>
<td>85.1-99.5</td>
<td>136/136</td>
</tr>
<tr>
<td>HHV-6</td>
<td>12/16</td>
<td>75.0</td>
<td>50.5-89.8</td>
<td>168/168</td>
</tr>
<tr>
<td>HPeV</td>
<td>2/3</td>
<td>66.7</td>
<td>20.8-93.9</td>
<td>187/187</td>
</tr>
<tr>
<td>VZV</td>
<td>22/22</td>
<td>100</td>
<td>85.1-100</td>
<td>162/164</td>
</tr>
<tr>
<td><strong>Yeast</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>C. neoformans/gattii</em></td>
<td>19/19</td>
<td>100</td>
<td>83.2-100</td>
<td>171/171</td>
</tr>
</tbody>
</table>
Contrived Study

- Residual CSF specimens negative for all ME panel analytes (by FilmArray and comparator methods) were co-spiked

- **235 Specimens**
  - 35 Negative
  - 50 specimens per analyte with at least six quantified isolates evaluated
    - 25 at 2 × LoD
    - 25 at four concentrations spanning the clinically relevant range (based on Cp values from previous FilmArray ME Panel positive test results)

- **Specimens tested at six of the prospective study clinical laboratories**
## Contrived Performance

<table>
<thead>
<tr>
<th>Analyte</th>
<th>/PPA</th>
<th>NPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP/(TP + FN)</td>
<td>%</td>
</tr>
<tr>
<td>E. coli K1</td>
<td>47/49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.9</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>50/50</td>
<td>100</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>50/50</td>
<td>100</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>75/75</td>
<td>100</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>48/50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>96.0</td>
</tr>
<tr>
<td>CMV</td>
<td>47/49&lt;sup&gt;c&lt;/sup&gt;</td>
<td>95.9</td>
</tr>
<tr>
<td>HHV-6</td>
<td>50/50</td>
<td>100</td>
</tr>
<tr>
<td>HPeV</td>
<td>50/50</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup> One *E. coli* false negative was observed at 2 × LoD and one *E. coli* false negative was observed at 0.2 × LoD.

<sup>b</sup> Both *S. agalactiae* false negatives were observed at 0.2 × LoD.

<sup>c</sup> Both CMV false negatives were observed at 0.2 × LoD.
Summary of Registration Studies

FilmArray ME Panel is:

- **Sensitive**
  - LoD in 100 – 1,000 copies/mL
  - Clinical study showed 95-100% PPA for most analytes

- **Specific**
  - Limited cross-reactivity
  - Clinical study showed >99% NPA for all analytes

- **Reliable**
  - Highly reproducible test results
  - Robust
  - Few errors/control failures in intended use setting