Dedication to Viral Pathogens in CSF by the FilmArray™ ME Panel

Elizabeth Ott, Seth Lilavatv, Jeffrey Nichols, and Stephanie Thatcher
BioFire Diagnostics, LLC, Salt Lake City, UT

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INTRODUCTION/BACKGROUND

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

MATERIALS AND METHODS

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

RESULTS

All organisms on the ME Panel were detected.
• Detection ranged between 10^1-10^5 for most organisms.
• 100% detection of multiple organisms within one sample. This study confirmed detection of 8 organisms simultaneously.
• Mechanical lysis by bead beating increased performance of the FilmArray ME Panel.
• RNA and DNA targets were effectively isolated from CSF for downstream detection in the FilmArray ME Panel.
• Concentration of CSF was not necessary for detection.
• A minimal CSF volume of 200 uL was sufficient for detection.
• Blood contamination of up to 50% in CSF did not hamper performance.
• CSF and PBST performed similarly in the FilmArray ME Panel.

CONCLUSIONS

A rapid detection (~60 mins) of pathogens in CSF by the FilmArray ME Panel will facilitate prompt, appropriate patient treatment and mitigate empirical antibiotic use, both of which benefit patient outcomes and antibiotic stewardship.
• The FilmArray ME Panel has not been CE-marked or US FDA-cleared for in vitro diagnostic use.

The FilmArray ME pouch has a fiber-optic containing freeze-dried reagents and plungers that dispenses liquids to the film portion of the pouch. This process consists of reactions for cell lysis (C), magnetic bead-based nucleic acid purification (D & E), first-stage multiplex PCR (f1 & g) and an array of 102, second-stage nested PCRs (i).

PCR primers are dried into the wells of the array and each primer set amplifies a unique product of the first-stage multiplex PCR. The second-stage PCR product is detected in a melting analysis using a fluorescent double-end ITDNA binding dye, LCGreen™.

The FilmArray System

The FilmArray is a lab-in-a pouch, medium-scale, fluid manipulation test performed in a self-contained disposable thin-film plastic pouch. The FilmArray platform processes a single sample, from nucleic acid purification to result, in a fully automated fashion.

Sample Processing and Pouch Loading Instruction

Step 1

Step 2

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

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

<table>
<thead>
<tr>
<th>Organism</th>
<th>14*</th>
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<th>14*</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>N. meningitidis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>S. agalactiae</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>H. parainfluenza</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fungi</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Cryptococcus gattii</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The FILMARRAY MENINGITIS/ENCEPHALITIS (ME) PANEL

Simultaneous detection of 18 targets.

Bacteria
- Escherichia coli K1
- Klebsiella pneumoniae
- Listeria monocytogenes
- Neisseria meningitidis
- Pseudomonas aeruginosa
- Streptococcus agalactiae
- Streptococcus pneumoniae

Viruses
- Cytomegalovirus
- Enterovirus
- Epstein-Barr virus
- Herpes simplex virus 1
- Human herpesvirus 6
- Human parainfluenza virus 1
- Varicella zoster virus

Fungi
- Cryptococcus neoformans
- Cryptococcus gattii

Figures 1, 2, 3, 4, 5

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

<table>
<thead>
<tr>
<th>ME Alc/ID (ID 170)</th>
<th>CSF Volume (uL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 uL</td>
<td>0.10 ± 0.00</td>
</tr>
<tr>
<td>200 uL</td>
<td>0.13 ± 0.00</td>
</tr>
<tr>
<td>300 uL</td>
<td>0.15 ± 0.00</td>
</tr>
</tbody>
</table>

Table 3. Minimal Volume of 200 uL Required for FilmArray ME

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<th>ME Alc/ID (ID 170)</th>
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<td>300 uL</td>
<td>0.15 ± 0.00</td>
</tr>
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Figure 6. 95% Confid. Interval (95% CI) for Sensitivity and Specificity

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>N. meningitidis</td>
<td>95% ± 2%</td>
<td>98% ± 2%</td>
</tr>
<tr>
<td>H. influenzae</td>
<td>97% ± 1%</td>
<td>99% ± 1%</td>
</tr>
<tr>
<td>H. parainfluenza</td>
<td>98% ± 1%</td>
<td>99% ± 1%</td>
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</table>

*Presented at the European Society for Clinical Virology, September 2014

Contact Information
BioFire Diagnostics
5500 W. 500 N.
Logan, UT 84321
801-673-8888
www.biofiredx.com