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# Preclinical Assessment of a Fully Automated Multiplex PCR Panel for Detection of Central Nervous System Pathogens

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**We evaluated a multiplexed PCR panel for the detection of 16 bacterial, viral, and fungal pathogens in cerebrospinal fluid. Panel results were compared to routine testing, and discrepancies were resolved by additional nucleic acid amplification tests or sequencing. Overall, the positive and negative agreements across methods were 92.9% and 91.9%, respectively.**

Acute meningoencephalitis (ME) is an inflammatory disease of the central nervous system (CNS) that can result in significant morbidity and mortality. Prompt diagnosis is essential for optimal outcomes (1–3) and resource utilization (4), but confirming an infectious etiology is often difficult and time-consuming. Culture remains the diagnostic gold standard for the diagnosis of bacterial and fungal ME, while nucleic acid (NA) amplification using PCR is used routinely for viruses.

BioFire Diagnostics (Salt Lake City, UT) has developed a fully automated, multiplexed PCR system called the FilmArray (FA) that can test for large combinations of infectious agents simultaneously. A FilmArray ME panel was designed to detect and identify 16 common bacteria, viruses, and yeasts directly from cerebrospinal fluid (CSF). The purpose of this study was to evaluate the test performance of a research only (RUO) version of the panel (Table 1) compared to that of conventional microbiologic testing.

(Portions of the this study were previously presented at the American Society for Microbiology Meeting, Boston, MA, 17 to 20 May 2014, and at the Infectious Diseases Society of America Meeting, Philadelphia, PA, 8 to 12 October 2014.)

CSF samples obtained by lumbar puncture between August 2012 and March 2014 were retrieved from frozen storage (−20°C) at the University of Utah's ARUP Laboratories and Primary Children's Hospital (Salt Lake City, UT). Specimens were eligible to be included in the study if (i) they had been previously analyzed with at least one conventional method (bacterial culture, viral PCR, and/or cryptococcal antigen [CrAG]) and (ii) there was an adequate residual volume for FA ME testing and discrepancy resolution testing if necessary. Only the first CSF specimen submitted with adequate volume per patient was included in the study repository. Specimens were linked to the routine microbiology results and then deidentified prior to FA ME testing.

FA ME testing was performed by investigators blinded to the conventional test results. The RUO multiplex panel was used per the manufacturer's instructions. Briefly, 200 μl of CSF was diluted 1:4 with sample buffer and was injected into a single-use FA ME pouch. Testing was performed on the commercially available FA instrument with RUO software. NA extraction, purification, amplification, and results interpretations are automated within the FA system. Assay run time was approximately 1 h with 5 min of hands-on work.

ARUP Laboratories-developed real-time PCR tests (LDTs) (5–9) were used to resolve specimens with viral NA detected by FA ME testing that had not been previously tested by PCR as a part of routine clinical care. The LDT assays use 200 μl of CSF to perform automated nucleic acid extraction on the chemagic MSM1 (PerkinElmer, Shelton, CT), fluorogenic hybridization probe PCR chemistry (10), and amplification/detection using the 7900 real-time PCR system (Applied Biosystems, Grand Island, NY). CSF specimens that had a virus detected by an LDT during standard clinical care but that were negative by FA ME testing were retested using a second commercially available real-time PCR assay (genesig kits; Primerdesign Ltd., Southampton, United Kingdom). CrAG testing was performed by enzyme immunoassay (EIA) (cryptococcal antigen enzyme immunoassay; IMMY, Norman, OK) with bidirectional 23S rRNA gene sequence analysis used to help adjudicate all *Cryptococcus* and/or bacterial discordant specimens. The bacterial culture and CrAG test results were considered to be the diagnostic gold standards irrespective of the DNA sequence analysis.

The sensitivity and specificity of the individual FA ME components were calculated using the conventional test or the discrepancy resolution LDT results as the diagnostic gold standard. FA ME only detections were considered to be true positives if they were confirmed by resolution testing. Agreement between conventional methods and the FA ME panel was assessed using the kappa (κ) statistic, and proportions were compared using the chi-square test in Analyze it version 4.2 (Leeds, United Kingdom) for Microsoft Excel 2010.

A total of 197 adult and 145 pediatric CSF specimens were retrieved for the study; there were 17 bacterial culture-positive,

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**TABLE 1** Distribution of organisms identified by conventional methods and the FilmArray meningitis/encephalitis (FA ME) panel

| Organism identification[a] | Conventional detection, no. | FA ME panel detection, no. | Baseline agreement, no. | Resolution result, no.[b] | | | Sensitivity, % (95% CI)[c] | Specificity, % (95% CI)[c] |
|---|---|---|---|---|---|---|---|---|
| | | | | FA+/R+ | FA+/R− | FA−/R+ | | |
| Bacteria | | | | | | | | |
| *H. influenza* | 4 | 5 | 4 | 1 | 0 | 0 | 100 (47.8–100) | 100 (97.4–100) |
| *S. pneumoniae* | 3 | 6 | 3 | 2 | 1 | 0 | 100 (47.8–100) | 99.3 (96.1–100) |
| *S. agalactiae* | 1 | 5 | 1 | 2 | 2 | 1 | 66.7 (9.4–99.2) | 98.6 (95.0–99.8) |
| *Escherichia coli* | 1 | 1 | 1 | NA[d] | NA | NA | 100 (2.5–100) | 100 (97.5–100) |
| *Listeria monocytogenes* | 0 | 0 | 1 | NA | NA | NA | NA | 100 (97.5–100) |
| *Neisseria meningitides* | 1 | 1 | 1 | NA | NA | NA | 100 (2.5–100) | 100 (97.5–100) |
| Bacteria not in the FA ME panel[e] | 7 | 0 | 7 | NA | NA | NA | NA | NA |
| Viruses | | | | | | | | |
| EV | 37 | 37 | 36 | 1 | 0 | 1 | 97.4 (86.2–99.9) | 100 (69.2–100) |
| HSV-1 | 12 | 13 | 11 | 0 | 2 | 1 | 92.9 (66.1–99.8) | 98.0 (89.1–99.9) |
| HSV-2 | 29 | 29 | 29 | NA | NA | NA | 100 (88.1–100) | 100 (82.4–100) |
| HHV-6 | 13 | 18 | 12 | 6 | 0 | 1 | 94.7 (74.0–99.9) | 100 (92.6–100) |
| VZV | 32 | 32 | 32 | NA | NA | NA | 100 (89.1–100) | 100 (79.4–100) |
| CMV | 7 | 4 | 4 | 0 | 0 | 3 | 57.1 (18.4–90.1) | 100 (91.4–100) |
| EBV | 13 | 25 | 11 | 5 | 9 | 1 | 94.1 (71.3–99.9) | 84.2 (72.1–92.5) |
| PV | 0 | 1 | 0 | 1 | 0 | 0 | 100 (2.5–100) | 100 (92.5–100) |
| Yeast | | | | | | | | |
| *C. neoformans/gattii* | 14 | 9 | 8 | 1 | 0 | 0[f] | 64.3 (35.1–87.2) | NA |
| Total | 174 | 186 | 161 | 19 | 14 | 8 | 92.8 (88.2–96.0) | 92.8 (88.2–96.0) |

[a] Organisms detected by the FA ME panel include *Escherichia coli K1*, *Haemophilus influenza*, *Listeria monocytogenes*, *Neisseria meningitides*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, cytomegalovirus (CMV), enterovirus (EV), Epstein-Barr virus (EBV), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human herpesvirus 6 (HHV-6), human parechovirus (PV), varicella-zoster virus (VZV), and *Cryptococcus neoformans/Cryptococcus gattii*. The FA ME assay does not differentiate cryptococcal species.
[b] FA, FilmArray; R, discrepancy resolution nucleic acid test result; +, positive result; −, negative result.
[c] Calculated sensitivity and specificity takes in to account discrepancy resolution testing. CI, confidence interval.
[d] NA, not applicable or not able to calculate.
[e] Bacteria isolated in culture but not included in the panel were *Citrobacter koseri*, *Klebsiella pneumoniae*, and *Providencia stuartii* as well as possible skin contaminants *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus warneri*.
[f] Six cryptococcal antigen-positive, FA ME-negative specimens were also negative for yeast by DNA sequence analysis.

128 bacterial culture-negative, 143 viral LDT PCR-positive, 48 viral LDT PCR-negative (for all targets included in the FA ME panel), and 14 CrAG-positive CSF specimens. The distribution of organisms identified as a part of routine clinical care is shown in Table 1.

The FA ME panel was negative for 14 pathogens detected by conventional testing (1 *Streptococcus agalactiae* specimen; 7 viruses—3 cytomegaloviruses [CMVs], 1 herpes simplex virus 1 [HSV-1], 1 human herpesvirus 6 [HHV-6], 1 Epstein-Barr virus [EBV], and 1 enterovirus [EV]; and 6 CrAG-positive specimens). The detection of *S. agalactiae* and the viral detections were confirmed by additional nucleic acid testing, but the 6 CrAG-positive/FA ME-negative specimens were also negative for cryptococcal DNA by sequencing. The multiplex panel generated 33 additional detections in specimens that were culture negative or not previously tested for the same pathogen as a part of routine testing. Nineteen of the additional FA ME detections were confirmed (2 *Streptococcus pneumoniae* specimens, 2 *S. agalactiae* specimens, 1 *Haemophilus influenzae* specimen, 1 *Cryptococcus* sp. specimen, 6 HHV-6, 4 EBVs, 1 HSV-1, 1 EV, and 1 human parechovirus [PV]). Following resolution testing, the overall agreement between conventional and FA ME testing for the targets included in the panel was 92.8% ($\kappa = 0.86$; $P < 0.0001$). In general, false-negative FA ME results came from specimens with relatively low CrAG titers (range, 1:4 to 1:116) and/or high PCR crossing thresholds (>36 cycles). More than half (70%, 7/10) of discrepant or unconfirmed FA ME-positive results were for EBV.

Identification of more than one organism by FA ME testing was observed in 6% (20/342) of specimens (Fig. 1). EBV was the most common organism identified as a part of dual detections (14/20), followed by HSV-1 or HSV-2 (8/20) and CMV (4/20). In all, 76.2% (32/42) of the FA ME codetections were confirmed or in agreement with baseline conventional results. EBV DNA that was detected by only the FA ME panel in 7 specimens accounted for the majority of unconfirmed codetections. Polymicrobial infection was more common in adult that in pediatric specimens (9% versus 2%; $P = 0.01$). The immunologic status of subjects was not known, and it is important to note that the significance of detecting latent or reactivated herpesviruses in CSF is often uncertain and will require clinicians to interpret panel results in the clinical context.

Our study has several important limitations. First, the sample size was relatively small, which impacted the statistical certainty of the FA ME assay sensitivity and specificity calculations. Not all specimens were tested by all methods, but we attempted to resolve discrepancies using additional NA testing. Although the ARUP Laboratories-developed viral PCR assays appeared to be slightly more sensitive than the multiplex panel, we did not directly compare the analytical limits of detection, and the effects of freeze-thaw cycles may have impacted FA ME sensitivity and/or the abil-

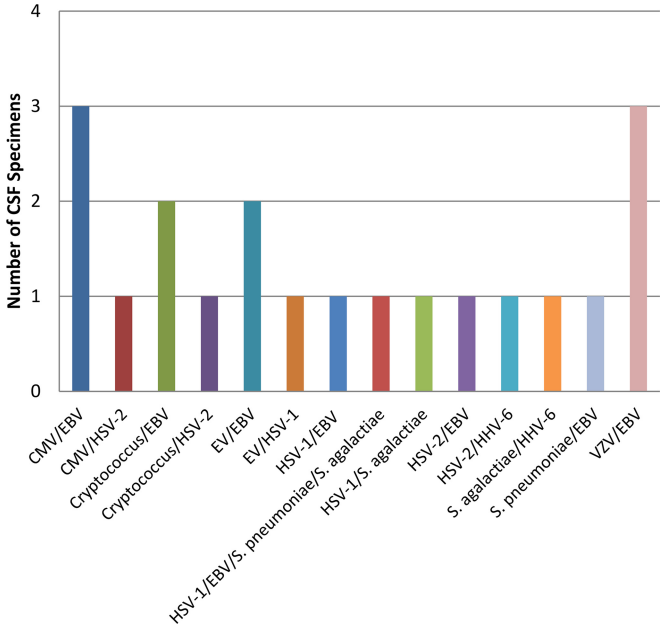


**FIG 1** Coinfections detected by multiplex PCR. *, Ten of the FilmArray co-detection results could not be confirmed with resolution testing (7 EBVs, 1 HSV-1, 1 *S. pneumoniae* specimen, and 1 *S. agalactiae* specimen).

ity to confirm previous results. CrAG testing is a highly sensitive and accurate method for diagnosing cryptococcal meningitis (11, 12), but we did not have fungal culture results to link to the FA ME and DNA sequence analyses. Furthermore, we did not have access to patient-level information to help adjudicate discrepancies across methods.

The RUO version of the FA ME panel showed near-perfect agreement with bacterial culture and viral PCRs (other than EBV and CMV) and was able to detect bacteria in several culture-negative CSF specimens, possibly after the administration of antimicrobial therapy. In contrast, there was less agreement with CrAG testing, which may be more sensitive than molecular methods and remains the preferred approach for the rapid diagnosis of cryptococcal meningitis.

The FA ME panel detects the most common causes of acute bacterial and viral ME that affect neonatal, pediatric, and adult patients (13, 14) and includes potential opportunistic pathogens, which are more likely to cause disease in immunocompromised hosts. The FA ME panel was recently approved by the U.S. Food and Drug Administration with the exception of the EBV target, which was removed from the final version of the test. Our preliminary work suggests that the FA ME panel can be a useful adjunct for the diagnosis of suspected CNS infection when used in combination with other tests, such as culture, CrAG, and/or pathogen-specific PCR.

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