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COMMENTARY

The First Fully Automated Molecular Diagnostic Panel for Meningitis and Encephalitis: How Well Does It Perform, and When Should It Be Used?

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Rapid and accurate molecular diagnostic tests for the most common causes of infectious meningitis and encephalitis have the potential for high clinical impact. In this issue of the *Journal of Clinical Microbiology*, Leber et al. (J Clin Microbiol 54:2251–2261, 2016, http://dx.doi.org/10.1128/JCM.00730-16) report results from a large clinical study designed to prospectively assess the performance of the FilmArray meningitis/encephalitis panel compared to conventional methods.

Meningitis and encephalitis are potential infectious disease emergencies. The laboratory evaluation of suspected meningitis/encephalitis (ME) is complex, in part because the differential diagnosis is broad and the associated clinical signs and symptoms are not organism specific. Clinicians typically prioritize laboratory workup based on host factors, the duration of symptoms, and potential environmental exposures; but cerebrospinal fluid (CSF) indices in combination with multiple microbiologic tests are generally required to exclude infection or identify a potential pathogen.

CSF Gram stain with culture has been the diagnostic "gold standard" for acute bacterial meningitis for nearly a century. However, both methods have a limited sensitivity that may be further reduced in patients who have received antibiotics (1). As a result of these limitations, there has been much interest in the development of standardized molecular diagnostic tests for bacterial central nervous system (CNS) infections. Molecular assays have the potential to be more rapid than culture and are potentially less affected by prior antimicrobial therapy. Until recently, there were no commercially available molecular assays for the diagnosis of bacterial ME marketed in the United States. In contrast, nucleic acid amplification tests are the diagnostic standard for most viral CNS infections. U.S. Food and Drug Administration (FDA)-approved PCR assays have been available for enteroviruses (Xpert EV; Cepheid, Sunnyvale, CA) and herpes simplex viruses (Simplexa HSV 1&2 Direct; Focus Diagnostics, Cypress, CA) for years. Some clinical laboratories have also developed their own PCR assays for CNS pathogens, while others may send testing out to a reference laboratory, which potentially delays time to actionable results.

In October 2015, the FDA cleared the first multiplex PCR panel for the detection of CNS pathogens. The fully automated FilmArray ME panel (BioFire Diagnostics LLC, Salt Lake City, UT) simultaneously detects and identifies 14 bacterial, viral, and yeast pathogens in about an hour directly from 200 μl of CSF. The components of the FDA-approved FilmArray ME multiplex panel are listed as follows. (i) The bacteria are *Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*. (ii) The viruses are cytomegalovirus (CMV), enterovirus (EV), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), human herpesvirus 6 (HHV-6), human parechovirus (PeV), and varicella-zoster virus (ZVZ). (iii) The yeasts are *Cryptococcus neoformans* and *Cryptococcus gattii*. (Note that the assay does not differentiate *C. neoformans* from *C. gattii*.)

In this issue of the *Journal of Clinical Microbiology*, Leber and colleagues report the results of a large multicenter trial designed to support FDA clearance of the ME panel (2). Residual cerebrospinal fluid (CSF) specimens obtained as a part of routine clinical care were tested at 11 different sites in the United States. The ME panel test performance was assessed through comparisons to conventional culture for bacteria or with PCR followed by sequencing for the viral and yeast targets. Discordant results across methods were resolved by repeat molecular analysis (when possible) combined with a blind review of study subject demographic, clinical, and laboratory information.

A total of 1,560 prospectively collected CSF specimens were enrolled in the study over 8 months. There was 84.4% positive and >99.9% negative agreement between the ME panel and conventional methods after adjudication of discrepant results. The ME panel missed 1 *Streptococcus agalactiae*-positive specimen, 2 EV-positive specimens, and 3 HHV-6-positive specimens. Unfortunately, there were too few cases of bacterial or cryptococcal meningitis diagnosed during the study period to make robust assessments of the bacterial and yeast components of the ME panel. Most of the viral targets showed excellent agreement with comparator PCRs, even though the multiplex format is slightly less sensitive than the comparator singleplex tests. A notable exception was the calculated sensitivity of the HHV-6 target (86%) and, in a previous report, the cytomegalovirus (CMV) target (57%) (3). Although the ME panel missed two enteroviral infec-

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tions, the majority of these infections were detected (44/46; sensitivity, 96%).

The most disconcerting result of the study was that there were as many false-positive or unconfirmed ME panel results ($n = 22$) as there were additional confirmed detections ($n = 21$) made by the multiplex PCR. The comprehensive list of targets included in the ME panel helps to ensure that an actionable diagnosis will not be missed, but the false-positive results are concerning. *Streptococcus pneumoniae* was the most frequent false-positive detection made by the ME panel. The investigators speculated that contamination during specimen handling, potentially as a result of carryover from the positive-control material or normal flora of the operators, could have accounted for some of these results. These observations highlight the importance of laboratory operating procedures designed to minimize contamination, even when using a closed system such as the FilmArray. Contamination control may not be practiced meticulously when testing is performed outside dedicated molecular sections of the laboratory. Therefore, consideration should be given to having operators wear a mask when loading the FilmArray pouches or ideally use a biological safety cabinet or dead air box that can be cleaned and kept separate from positive-control materials. Establishing expected positivity rates for the individual targets contained in the ME panel will be an important way to monitor for contamination. It is important to note that the study design did not necessarily select subjects with a high pretest probability of CNS infection, which would also decrease the positive predictive value of ME panel results in some cases.

The authors are to be commended for reviewing a significant amount of patient-level data to help determine the accuracy of the ME panel. These efforts show how hard it can be to retrospectively reconcile unexpected molecular results with an imperfect diagnostic gold standard (i.e., Gram stain and culture) and with limited clinical information and/or no remaining specimen for retesting in some instances. Additionally, ME is a relatively rare condition. Despite conducting a large prospective study, statistically significant sensitivity and specificity calculations could be reported for only 9 of the 14 targets in the panel.

As syndromic "one size fits all" diagnostic panels become more common in clinical practice, laboratorians will be faced with guiding the rational use of these expensive technologies in the absence of cost-effectiveness studies. FDA approval does not necessarily mean that the ME panel will be the right test for all patients. Several factors should be considered when contemplating implementation of the ME panel. First, which patients should be tested? The organisms targeted by the ME panel make the most clinical sense for immunocompromised hosts, a setting where multiple *Herpesviridae* and *Cryptococcus* species can cause significant disease. In pediatric and adult patients with a high clinical suspicion for bacterial infection, the ME panel could also speed time to diagnosis and may be especially useful in situations where patients have received antibiotics before the diagnostic lumbar puncture is performed. Outside of these selected situations, however, targeted testing with prioritization of likely pathogens should be considered first. Laboratory acceptance criteria that are based in part on CSF nucleated cell counts could be considered a way to minimize unnecessary testing for immunocompetent adults, for example (4).

Next, laboratory consultation services will be needed to help clinicians interpret unexpected ME panel results. False-positive CSF test results have the potential to cause significant harm if they lead to the administration of unnecessary, potentially toxic treatment or unwarranted invasive procedures. Several of the false-positive ME panel results in this study could have theoretically had significant consequences if they were acted on (i.e., the 2 unconfirmed *Cryptococcus*, 9 bacterial, 4 HSV, and 2 CMV detections). Alternatively, a negative ME panel result does not exclude infection due to organisms that are not included in the panel, and false-negative results for targeted pathogens that are present in low quantities is still possible. Empirical antibiotics and/or acyclovir should still be administered when the clinical suspicion for bacterial infection or herpes simplex virus (HSV) encephalitis is high and the ME panel is negative. Finally, deciphering the clinical significance of reactivated or latent *Herpesviridae* can be challenging. Providers must consider these results carefully in the clinical context.

There is another issue for laboratories to consider: can the ME panel replace any of our current assays? Unfortunately, the answer to this question is probably "no." Gram stain remains essential for interpreting the PCR results, while CSF and blood culture are required to detect organisms not targeted by the ME panel as well as to have an isolate for susceptibility testing. The ME panel cannot replace the CSF cryptococcal antigen (CrAg). CrAg testing is fast, cheap, more sensitive, and probably more specific than PCR for the diagnosis of cryptococcal meningitis (3, 5–7). Whether the ME panel can replace individual viral PCR assays is questionable. I would argue that the ability to perform targeted testing based on clinical suspicion is likely to be more cost-effective for immunocompetent patients, especially when the testing can be performed in-house. In the end, the ME panel looks to be an additional test that does not necessarily replace current alternatives.

Large multiplexed panels represent a paradigm shift for medical microbiology and clinical infectious diseases alike. The study by Leber and colleagues represents the largest clinical assessment of a molecular panel designed to aid in the diagnosis of meningoencephalitis. This study, combined with previous retrospective reports, suggest that ME panel test characteristics are acceptable for clinical care (2, 3, 7). The main benefit of the ME panel is the potential for more rapid results, which could help inform optimal therapy and resource utilization. However, due to the potential for contamination combined with the ability to detect latent or reactivated viruses, ME panel detections must be scrutinized carefully, with positivity rates monitored closely by the laboratory. Future implementation research should focus on the potential clinical impact of a more rapid, comprehensive diagnosis of meningoencephalitis. In the meantime, it is essential that local implementation of the ME panel be done in partnership with clinicians to ensure that there is a clear understanding of test characteristics, result interpretation, and appropriate test utilization.

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