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POINT-COUNTERPOINT

Point-Counterpoint: Meningitis/Encephalitis Syndromic Testing in the Clinical Laboratory

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INTRODUCTION Syndromic panels were first FDA cleared for detection of respiratory pathogens in 2008. Since then, other panels have been approved by the FDA, and most recently, the FilmArray meningitis/encephalitis panel (BioFire, Salt Lake City, UT) has become available. This assay detects 14 targets within 1 h and includes pathogens that typically cause different manifestations of infection, although they infect the same organ system. Several studies have reported both false-positive and false-negative results with this test, and all agree that the cost is significant. As with other panels, health care systems have adopted different strategies for offering this assay. Some have implemented strategies to limit the use of the test to certain patient populations, others have elected not to offer the test, and others have elected not to offer the test and instead request that providers order specific PCRs for the pathogens that best fit the patient's symptoms. In this Point-Counterpoint, Jennifer Dien Bard of the Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, and of the Keck School of Medicine at the University of Southern California explains why laboratories should offer these assays without restriction. Kevin Alby of the University of Pennsylvania explains the concerns about the use of these assays as first-line tests and why some limitations on their use might be appropriate.

KEYWORDS *Neisseria meningitidis*, BioFire, encephalitis, enterovirus, film array, herpes simplex virus, lab utilization, *Streptococcus pneumoniae*

POINT

I'm not telling you it's going to be easy. I'm telling you it's going to be worth it.
—*Art Williams*

For the most part, syndromic testing by multiplex molecular panels has been a welcome addition to diagnostic laboratories. The first three panels available addressed syndromes associated with upper respiratory tract infections, bloodstream infections, and gastroenteritis, with adoption of the first two panels being the most widespread. Multiple studies have been published on the performance characteristics of these syndromic panels as well as the impact on turnaround times (TAT), increased pathogen detection, and antimicrobial use. Schreckenberger and McAdam previously published an excellent Point-Counterpoint article on the use of respiratory and gastrointestinal multiplex PCR panels as first-line testing (1). The focus of the debate was when to use the panels, rather than if the panels should be used at all.

In 2015, the Food and Drug Administration (FDA) cleared the first molecularly based syndromic panel for the simultaneous detection of multiple pathogens from cerebrospinal fluid (CSF) samples obtained via lumbar puncture. The FilmArray meningitis/encephalitis (ME) panel from BioFire Diagnostics has been met with polarized responses

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similar to those to cilantro—you either love it or hate it. Concerns have been raised over the potential for contamination associated with users' respiratory flora, as well as potential suboptimal sensitivity for certain targets. Moreover, the multicenter study suffered from a low number of overall positive results (8.7%), with detection of three of the herpesviruses and all bacterial targets other than *Streptococcus pneumoniae* being either sparse or nonexistent.

My intention is not to dismiss the limitations associated with the FilmArray ME panel because there are inarguable flaws. Rather, I contend that despite these limitations, the FilmArray ME panel (and other future multiplexed panel tests for pathogens in CSF) is a paradigm shift for many laboratories, and the potential impact that it can have on the diagnosis and management of patients with infections of the central nervous system (CNS) is substantial.

What is being offered and why is a game changer. Aside from 1 to 2 target assays to detect CNS infections caused by herpes simplex virus (HSV) and enterovirus, the FilmArray ME panel is the only FDA-cleared test offering rapid and simultaneous molecularly based detection of common etiologic agents of meningitis and encephalitis within 1 hour. The panel has 14 targets, consisting of 6 bacterial targets, namely, *Haemophilus influenzae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *S. pneumoniae*, *Streptococcus agalactiae*, and *Escherichia coli* K1; 7 viral targets, namely, herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), varicella zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus 6, (HHV-6), enterovirus, and human parechovirus (HPeV); and 1 fungal target, *Cryptococcus neoformans* or *Cryptococcus gattii*.

The FilmArray ME panel detects pathogens that are common in patients with community-acquired or perinatally acquired CNS infections, as well as pathogens that are more prevalent in an immunocompromised host, including CMV, HHV-6, and *Cryptococcus* spp. It would also be remiss not to acknowledge the testing restriction on patients with indwelling devices, as a different subset of pathogens are responsible for shunt infections. Thus, in the right patient population, this syndromic testing approach negates the initial requirement for *a priori* knowledge of potential infectious agents to reflect ordering practices. This is most evident in cases of viral infections where standard-of-care testing as part of the initial infectious disease workup may be limited to only HSV and enterovirus PCR, missing the more esoteric viral targets (HHV-6, CMV, and HPeV) that may not be high on the list for the differential diagnosis (2, 3). A prime example of this was described in a multicenter retrospective analysis of 145 neonates in which 17 additional pathogens were identified by FilmArray ME alone; HPeV PCR was not ordered for 7 of the 11 HPeV-positive cases detected by the FilmArray ME panel (4).

Identification of infectious etiologies improves patient management. Meningitis and encephalitis represent complex and severe syndromes that can progress rapidly in previously healthy individuals and are associated with high morbidity and mortality. The rates of bacterial meningitis have steadily declined in the United States since pneumococcal, meningococcal, and *H. influenzae* type B vaccine implementation, with $<$5,000 cases reported annually between 2003 and 2007 (5). Nonetheless, the case fatality rates hold steady, and prompt recognition of at-risk patients is imperative.

Patient management and outcomes are highly pathogen dependent, ranging from self-limiting and supportive therapy in many cases of enterovirus infections to severe complications and prolonged antimicrobial therapy in cases of bacterial or fungal pathogens or viral HSV, CMV, or VZV infections. The case fatality rate of bacterial meningitis (8.0 to 12.2%) is significantly higher than for viral meningitis ($<$1.0%) (5, 6), and an unfavorable outcome of bacterial meningitis directly correlates with delays in the initiation of adequate parenteral therapy (7). At Children's Hospital Los Angeles (CHLA), we encountered a case of culture-negative neonatal listeriosis detected solely by the FilmArray ME panel. In the absence of the molecular panel, adverse patient outcome may have resulted from parenteral therapy with ceftriaxone, which is ineffective against *L. monocytogenes* (8). A study of a small cohort of CSF-positive patients from Froedtert Hospital in Wisconsin reported a 15.4-h decrease in the time to targeted

therapy after implementation of the FilmArray ME panel. They also reported a decrease in 30-day mortality from 12.1% to 4.8% in the postimplementation group (9), although this decrease was not statistically significant.

Timely diagnosis of CNS infection or confidently ruling out the most common infectious processes also has a direct impact on reducing health care costs related to prolonged lengths of stay (LOS), extended therapy, and additional investigative procedures. For instance, a lower severity of disease and the absence of antimicrobial therapy required for most cases of aseptic meningitis significantly shortens the LOS (mean LOS = 3.7 days) compared to that for bacterial meningitis (mean LOS = 16.6 days) (6). In neonates with known viral infections, the median LOS was 44 h compared to 72 h when no viral etiology was identified by conventional testing (4). Preliminary data from Baystate Health also noted shortened LOS by 2.9 days for patients positive for a viral target from a FilmArray ME panel compared to that for negative patients. They also reported an additional 19/24 viral pathogens (71% of samples were positive for HPeV) that would have been missed had they continued to offer only an enterovirus PCR (11).

Although rare, there have been reports of concomitant bacterial and viral infections. A French group reported two cases (1.3%) of concurrent enteroviral and bacterial meningitis (with *S. pneumoniae* and *N. meningitidis*), highlighting the potential risks of initial screening by single-target PCR assays (12). A feature of the FilmArray ME panel that ameliorates this risk is the inclusion of bacterial, viral, and fungal targets. Data from 251 pediatric patients at CHLA showed that the FilmArray ME panel was an accurate predictor of negative results, with no additional on-target positives detected by further testing of CSF samples (unpublished data). Thus, implementation of the test alongside effective antimicrobial stewardship strategies can maximize the impact of the test on antimicrobial utilization. Preliminary analysis of 135 patients at CHLA found that 41.7% of patients positive with a viral target by the FilmArray ME panel had antibiotics discontinued in <24 hours compared to 5.0% of patients negative for all targets (13). Kim et al. also reported a significant decrease in the duration of antimicrobial therapy by approximately 48 h in cases where the FilmArray ME panel contributed to the medical decision-making (14).

Traditional diagnostics have significant shortcomings. Culture suffers from low sensitivity, particularly for patients with antimicrobial exposure prior to lumbar puncture (15). Unfortunately, it is not uncommon for cases of CNS infections to remain unresolved due to lack of an etiologic diagnosis (16), inevitably leading to potentially unnecessary antimicrobial therapy. The FilmArray ME panel may circumvent this sensitivity issue, allowing for identification of a potential pathogen even when it is nonviable in culture due to initiation of parenteral therapy. To emphasize, there are currently no other FDA-cleared tests for the detection of bacterial pathogens from CSF. This means that prior to the FilmArray ME panel, there were limited options available to clinicians desperate to identify a definitive etiology in the absence of a positive culture. Small numbers of studies and case reports have noted increased detection of bacterial targets by the FilmArray ME panel alone (8, 17–20). A study of 62 CSF samples from young infants tested by the FilmArray ME panel reported 7 more positive results for bacterial targets than found with culture; 7 of the positive results were from infants that received antibiotics prior to lumbar puncture (18). Within the first 4 months of implementation, the FilmArray ME panel identified two cases of culture-negative bacterial meningitis (*S. pneumoniae* and *L. monocytogenes*) that were confirmed by positive blood cultures (14). Similarly, data from 1 year of testing at CHLA on 251 patients noted that only 3/8 specimens positive for bacteria were positive by culture (unpublished data). We also reported 2 cases of FilmArray ME panel-positive, culture-negative bacterial meningitis in patients with no prior antimicrobial exposure (8, 17). In 5/7 additional cases, the FilmArray ME panel result was supported by one or more of the following: abnormal CSF parameters and confirmatory molecular testing. The inadequacy of conventional culture is corroborated by the aforementioned culture-negative cases, emphasizing the need for novel, molecular tests to assist in the diagnosis of CNS infections.

The timely availability of diagnostic results is critical, as it can directly influence medical decision-making. Compared to culture or conventional PCR assays, the FilmArray ME panel is very fast, with 2 min of hands-on time and 1 h of run time, which makes it conducive to a continuous testing approach. Other molecular options, such as targeted sequencing and metagenomic next-generation sequencing, offer broad-range detection but come at a high cost and delayed TAT, minimizing the real-time impact. The average TAT of the FilmArray ME panel at CHLA is 3.1 h, with the results sometimes available prior to completion of CSF Gram staining. Similarly, a dramatic reduction in median times to organism identification from 119 to 3.5 h was recently reported (9).

Widespread availability in community hospitals. Many laboratories lack the appropriate infrastructure or personnel to offer molecular testing in house. For these laboratories, all viral PCRs are sent out to a reference laboratory, resulting in significant delays in TAT. In multiple instances, patients will remain on acyclovir therapy or other antiviral agents until the viral PCR results are back, adding to the potential risk of adverse effects, such as neurotoxicity and renal dysfunction (21).

For these institutions, the availability of panels such as the FilmArray ME assay mitigates the delays caused by sending specimens to reference laboratories and allows hospital laboratories to provide expedient testing around the clock. The impact of this is profound and directly uplifting for providers with growing wariness caused by a limited testing menu. Investigators recently described the positive impact of bringing in the FilmArray ME panel in lieu of sending samples for external testing (22). In addition to noting increased target detection and decreased inpatient stays, the investigators noted a significant cost reduction of $360/test, which equates to a cost savings of $78,956 ($n = 243$) in the 2nd year of implementation.

Improved detection of unexpected etiologies. The signs and symptoms of CNS infections can be nonspecific, and the targets included in the FilmArray ME panel are not commonly investigated when conventional diagnostic approaches are used. Since implementation of the panel at CHLA, multiple diagnoses of HHV-6 meningoencephalitis have been made; FilmArray ME panel findings were corroborated by leptomeningeal enhancements and detection of decreasing HHV-6 titers in whole blood. We also recently identified a case of CMV meningoencephalitis, which prompted an ophthalmology consultation and subsequent diagnosis of CMV retinitis. In the absence of the FilmArray ME panel, diagnosis might have been delayed or entirely missed, as HHV-6 and CMV infections were not suspected in either case.

That being said, due to the potential for chromosomal reintegration and reactivation, detection of HHV-6 raises a diagnostic conundrum that can result in misdiagnosis and unnecessary exposure to antiviral agents. Correlation of an HHV-6-positive result with clinical findings, as well as investigation of the titer of HHV-6 in blood, is necessary to rule out HHV-6 reactivation or HHV-6 chromosomal integration (23).

Powerful tool when corroborated with clinical findings. Now let us discuss the elephant(s) in the room. One of the noted pitfalls of the FilmArray ME panel is the increased risk for contamination, which may lead to misdiagnosis and poor management. The clinical trial data showed a high number of false-positive *S. pneumoniae* identifications with remnant CSF samples (24). An unfortunate case of a delayed diagnosis of tuberculosis was also recently reported due to detection of HSV-1 by the FilmArray ME panel (25).

Yes, contamination is an issue but can be alleviated by meticulous handling of specimens during collection and laboratory workup. Due to the simplicity of performing the test, the molecular nature of the test may be an afterthought for laboratory personnel. It is highly recommended that rigorous procedures be put in place to mitigate the risks of contamination. This may include careful handling of a specimen upon receipt in the laboratory, frequent glove changes, dedicated lab coats, and decontamination of the biological safety cabinet and FilmArray holder before and after testing. If possible, a dedicated biological safety cabinet for molecular testing with frequent wipe tests (monthly to quarterly) is beneficial to further alleviate potential contamination.

There are also concerns related to the suboptimal sensitivity to certain targets, including HSV-1 and *Cryptococcus neoformans* or *C. gattii*. Retrospective studies have revealed discrepant HSV-1 results: 3 false-negative results for CSF samples (26, 27), 2 of which were spun prior to being tested (26). In contrast, Messacar et al. (28) reported an additional case of HSV-1 positivity detected by the FilmArray ME panel that correlated clinically. These discrepant findings are likely due to a low viral load in the CSF as well as the limit of detection of the comparator assay, emphasizing the need for prudent verification studies prior to implementation. Prospective clinical testing of 21 CSF samples with the FilmArray ME panel and an alternate FDA-cleared PCR assay yielded 100% concordant negative results within our institution (unpublished data).

The clinical trial data raised concerns about *Cryptococcus* detection, as 7 patients that were FilmArray ME panel negative were found to be cryptococcal antigen (CrAg) positive (24). Of note, culture and comparator testing were also negative for *Cryptococcus*. A case of delayed diagnosis of cryptococcal meningitis in an immunocompromised patient due to two negative FilmArray ME panel results (29) was recently reported. The diagnosis was finally made by CrAg testing. This case highlights an extremely important point related to ordering practices to rule in or out potential pathogens. As with CSF culture, the FilmArray ME panel is not a standalone test, and additional testing may be necessary to rule out certain infections. Inarguably, CrAg testing (of serum and CSF) is an essential diagnostic tool for the diagnosis of cryptococcal infections (30). Thus, with patients for whom the suspicion for such infections is high, it is imperative that antigen testing be performed alongside culture and/or the FilmArray ME panel.

As with every *in vitro* diagnostic test offered in clinical laboratories, clinical correlation is imperative and infectious disease diagnosis should not be based solely on a laboratory test result. This is extremely important in the case of syndromic CNS testing but is also relevant for molecular testing for such infections as streptococcal pharyngitis and *Clostridium difficile*, for which detection of asymptomatic carriers is common. Thus, with the paradigm shift created by these new technologies, collaboration between the laboratory and clinicians is more crucial than ever to ensure that the most accurate results are being reported. I suggest that prior to reporting a positive FilmArray ME panel result, the result should be scrutinized carefully and the medical chart reviewed. Repeat testing may be helpful, but a repeat negative result may not necessarily negate the initial positive result in cases of low pathogen loads.

The FilmArray ME panel has the potential to revolutionize diagnostic testing for CNS infections. I believe that it is one of the more exciting syndromic panels to become available in the past few years and has opened the door for many laboratories to offer state-of-the-art molecular testing in real time. It also simplifies the ordering process for providers and ensures detection coverage of a variety of targets that may not necessarily be investigated. The concerns associated with the use of this test are valid, but rather than boycotting the test entirely, some suggestions are to ensure prudent molecular testing protocols, implement potential algorithms or testing restrictions, promote better communication between disciplines, and always correlate results in the context of clinical findings.

I understand that because we are dealing with such potentially severe syndromes, the stakes are very high, warranting a naturally cautionary response. But I argue that it is because the stakes are so high and the potential impact so great that my enthusiasm for the FilmArray ME panel remains high.

Jennifer Dien Bard

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COUNTERPOINT

It is easier to prevent bad habits than to break them.

—Attributed to Benjamin Franklin

Syndromic, panel-based testing has revolutionized the way microbiology is practiced. Culture for respiratory viruses, once performed routinely in clinical laboratories, is now largely the domain of public health, reference, and other specialized laboratories. Furthermore, the ability of these panels to be performed on small, fully automated sample-to-answer platforms has allowed them to be performed outside the

setting of a dedicated molecular laboratory. This, in turn, has brought molecular testing to the masses, allowing hospitals that had previously only sent out samples for molecular testing to test in house. Not only does this help hospital finances by bringing expensive molecular testing in house, but it also greatly reduces test turnaround times. In some cases, rapid syndromic testing is even able to impact the patient (1) by improving things such as antibiotic utilization.

The identification of a causative agent of ME is rare. One facet that cannot be understated is our limited experience with any effective testing for meningitis/encephalitis (ME), due to the relative scarcity of positive results for the disease that it detects. During any given respiratory season, we reach nearly 50% positivity with molecular methods during the peak of the season. We have predictable 4 to 5% positivity for bacterial gastroenteritis. For CSF specimens, we identify a causative agent for $<$1% of the hundreds of cases tested per year. We more frequently grow single colonies of questionable significance than we do true bacterial pathogens. In the clinical study of the FilmArray ME panel with over 1,500 specimens, only 104 were positive by reference methods for any target and 46 (44%) were enterovirus (2). As a comparison, in the clinical study for the gastrointestinal panel with a similar number of clinical samples tested, there were nearly 10 times (957) the number reference method-positive samples (3). For the 22 targets assessed, 16 had at least 10 positive reference samples. For the ME panel, only 3 of 14 targets had at least 10 positive reference samples. For the targets associated with high morbidity and mortality for which therapeutic options exist, a total of 15 positive samples were tested, 9 of which were bacterial/fungal, 2 of which were HSV-1, and 4 of which were VZV. There were no clinical samples positive for *L. monocytogenes* or *N. meningitidis*, with one sample each positive for *H. influenzae*, *S. agalactiae*, and C*ryptococcus*, two positive for *E. coli* K1, and four positive for *S. pneumoniae* by reference culture. Although the point sensitivity for the ME panel was good (85.7% to 100%), the lower bound of the 95% confidence interval was below 75% for 8/9 targets for which there were enough positive results to calculate a confidence interval (a minimum of 2 positive samples were needed for confidence interval calculation). Unlike with other types of syndromic panels with robust data and ample numbers of positive samples tested (3), the determination of performance characteristics is based largely on contrived and simulated samples that are potentially not very reflective of the samples that we see with our patients. Furthermore, the heavy use of contrived and simulated samples makes it harder to assess the true rate of contamination or false-negative results. This makes it even more important to carefully evaluate the test with an adequate number of samples from one's own lab before making the decision of whether or not to implement the test.

Is the more sensitive test a better test? Discrepant investigation is a key part of any analysis of a molecular assay, especially when it is compared to potentially less sensitive methods, such as culture. In the case of the ME panel, there were 6 potential false-negative results and 43 potential false-positive results (2). Discrepant resolution agreed with the gold standard in 5/6 cases of false-negative results and 22/43 cases of false-positive results. For targets with high morbidity and mortality for which therapeutic options exist (bacteria/fungi, HSV-1, and VZV), one false-negative result (*S. agalactiae*) resolved the discrepancy in agreement with the gold standard. Ten potentially false-positive ME panel detections (1 *H. influenzae* identification and 5 *S. pneumoniae*, 2 *Cryptococcus*, and 2 VZV identifications) were resolved against the gold standard, whereas 14 (1 *E. coli*, 1 *S. agalactiae*, and 1 VZV identification and 7 *S. pneumoniae*, 2 *Cryptococcus*, and 2 HSV-1 identifications) resolved the discrepancy in agreement with the gold standard. This suggests that specimens positive by PCR (or whatever you decide to call the test) but negative by conventional testing are equally or more likely to be truly false positive than really positive, which can have significant impacts on downstream decisions and potentially contribute to missed/delayed diagnoses. In fact, Gomez and colleagues described a case of delayed diagnosis of tuberculosis meningitis because of a false-positive HSV-1 result (4). The common argument

for adoption in the face of data showing the high rates of false-positive tests is that close attention to molecular practices will mitigate potential contamination issues. To that argument I pose this question, which is the more controlled setting: a clinical trial laboratory or your own laboratory? Our experience is that testing for any analyte is much more controlled during a trial where a couple of key users are trained and responsible for testing in a dedicated area than during routine use of an assay in the middle of our laboratory.

What do published data outside the primary clinical study say? Hanson and colleagues (5) evaluated the investigational use only (IUO) version of the ME panel, which included Epstein-Barr virus (EBV), a target that was not included in the version cleared by the FDA. The Hanson et al. study had smaller numbers and was biased toward previous positive results (160/342 were known to be positive, 143 of which were viral); however, they demonstrate some of the same issues that were seen in the larger Leber et al. (2) study: false-positive detections of significant targets (1 *S. pneumoniae* result, 2 *S. agalactiae* results, and 2 HSV-1 results), though with potentially more false-positive resolutions in agreement with the ME panel assay (1 *H. influenzae* result, 2 *S. pneumoniae* results, 2 *S. agalactiae* results, and 1 *Cryptococcus* result). Graf and colleagues (6) also performed a pediatric study biased toward positive results (67/133 samples were positive). In their study, the ME panel missed 2/38 enterovirus, 1/15 parechovirus, and 2/4 HSV-1 etiologies. Importantly, Graf et al. also reported on the threshold cycle (C_T) values of the missed targets. The missed enterovirus samples were originally detected at approximately cycles 39 and 42 (out of 45), with parechovirus having an original C_T of approximately 30 (out of 45). The HSV-1 samples were positive at approximately cycles 35 and 36 (out of 45). This indicates a wide range of viral burden that could be missed by the ME panel. Whether this translates into missing clinically significant infections cannot be assessed with contrived samples.

What is the clinical utility of the viral targets on the panel? In addition to the concern over potential false-positive target detection is the concern over the detection (either true or false) of a target with unknown clinical significance. Like other herpesviruses, HHV-6 is commonly encountered in childhood and develops latency. In one study, it was found in 37% of normal brain tissue samples (7). In the clinical evaluation of the ME panel, HHV-6 was the second-most-frequent positive result. Confounding matters further is that approximately 1% of the population has chromosomal integration of HHV-6 into their germ line genome (8). That being said, HHV-6 (primarily reactivated infection) is associated with encephalitis in adults, though primarily in hematopoietic stem cell transplant and other immunocompromised patients. A recent review of the literature found 45 articles on cases of HHV-6 encephalitis in adults, and the majority (22 hematopoietic stem cell transplantation [HSCT], 5 solid-organ transplantation [SOT], 3 HIV, and 5 medication patients) had some type of immunosuppression (9). Taken together, this makes interpretation of HHV-6 detection difficult, especially in a qualitative assay with no indication as to the amount of target present (e.g., the C_T value).

Similarly, CMV is another herpesvirus that has high seroprevalence and a predilection for latency in white blood cells (WBC). This presents a particular challenge in interpreting positive results for adult patients with pleocytosis, as the positive target might be attributed to detection of latent virus in WBC present in the CSF and not to active replicative virus causing symptoms (10). In general, syndromically based testing provides immense utility when performed on the correct patient population, as one test can answer many questions, preventing duplicative testing. Panel-based testing for meningitis/encephalitis, however, may be an exception, especially because the clinical presentations vary wildly depending on which patient and what syndrome (meningitis versus encephalitis) and offending agent (viral versus bacterial/fungal) are being tested. The combination of viral and bacterial/fungal targets provides two different, nonoverlapping use cases in one test. The first is for viral meningitis or encephalitis, a commonly encountered disease where some targets have well-described significance (HSV, VZV,

enterovirus) and others a more difficult interpretation (CMV, HHV-6), and many therapeutic decisions can be made on the basis of the results. It should also be noted, that there are still many causes of viral encephalitis, such as the arboviruses, that are not detected by this panel and would require additional testing to diagnose.

What is the significance of the bacterial targets on the panel? The second use case is for bacterial meningitis, a relatively infrequent disease in the postvaccine era which requires immediate attention. Unfortunately, while a large number of cases of bacterial meningitis are for organisms included in the panel, the risk of harm for any bacterial pathogen is significant enough that antibiotic therapy would persist even with a negative test. In fact, surveillance data from 2010 suggest that the incidence of staphylococcal and Gram-negative organisms (excluding *N. meningitidis* and *H. influenzae*) as the causative agents of bacterial meningitis trails only that of *S. pneumoniae* in the United States (11). Therefore, culture confirmation would likely be required before any modification of antibiotic therapy, limiting the value of negative bacterial targets. Additionally, the current panel lacks targets typically associated with device-associated infection, such as *Cutibacterium* (formerly *Proprionibacterium*) *acnes* or coagulase-negative *Staphylococcus* spp. Furthermore, given the low prevalence of bacterial meningitis, there may be as many false-positive results as true-positive results. The true difficulty, however, is the interpretation of the unexpected positive-a viral etiology of encephalitis in the setting of the clinical presentation of bacterial meningitis or vice versa. Finally, there is a significant variable of the patient population in which this test is used. Children may have more significant disease that would support treatment of a questionable positive result, whereas immunosuppressed adults may have more positive tests of unknown significance. For example, in the clinical study, nearly 40% of total samples and 66% of positive samples came from pediatric patients.

Conclusions. Ultimately, while the discussion itself about syndromic testing for meningitis/encephalitis is beneficial, the widespread adoption of this approach is premature. We simply do not yet have the data to know the significance of this testing. There will be cases where the utilization of a rapid syndromic panel allows for earlier targeted therapy and improved outcomes. However, based on the data available so far, it is just as likely, if not more likely, that incorrect diagnoses will be made based on erroneous results. Unfortunately, we do not yet have the data to determine in which settings this approach will be beneficial and in which settings it will be harmful. The danger in any syndromic panel is in thinking that the panel is the only test needed for infections associated with that disease process and that the answer, positive or negative, is definitive. This way of thinking could have catastrophic consequences if applied to meningitis/encephalitis testing. Unfortunately, we will not know the answer to the utility question without real-world experiences; however, we can mitigate the potential harm by ensuring that testing occurs only for the proper patients in the proper settings. Finally, it is critical that any testing be performed under careful observation and that use of the assay is prospectively evaluated for utility to prevent any "bad habits" from forming.

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SUMMARY

Points of agreement

- Syndromic panel testing has greatly reduced the turnaround times of molecular results and increased the diversity of laboratories that can perform testing.
- The clinical utility of HHV-6 and CMV on the FilmArray ME panel needs to be strongly correlated with patient history and underlying immune status.
- Inclusion of targets such as human parechovirus will increase the incidence of identification of these targets and may provide a better indication of their frequency of isolation.
- Identification of contaminants should be carefully monitored when using a meningitis/encephalitis panel. Possible solutions may include chart review of positive patients by the laboratory director or designee, strict disinfection protocols, and frequent monitoring for environmental contamination.
- More data on the performance of the FilmArray ME panel on prospective clinical specimens and the potential outcome benefits are needed.

Points requiring further consideration

- Studies evaluating the analytical performance of the FilmArray ME panel have extensively relied on contrived specimens and archived specimens. This has contributed to a positive bias in the literature, potentially overstating the performance of the ME panel.
- What is the most appropriate patient population for testing with the FilmArray ME panel?
- Outcome studies assessing the clinical utility of ME panel results are needed to assist clinical laboratories in determining the ideal approach for testing.

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