ME multiplex panel: debating the tradeoffs

Anne Paxton

June 2019—Meningitis and encephalitis have been called by some the most terrifying diseases in medicine, in part because of the difficulty of diagnosing their underlying pathology. The clinical stakes of laboratory testing are high for diagnosing and treating the inflammation that meningitis/encephalitis (ME) causes within the central nervous system. While an FDA-approved panel exists for molecular testing of cerebrospinal fluid for viruses, bacteria, and one fungus known to be associated with ME, questions surround the panel's use.

BioFire's FilmArray ME panel, which detects 14 targets directly from CSF obtained by lumbar puncture, is one of the most recently approved syndromic panel-based tests. But as the causes of ME in different patient populations have shifted in recent years, studies are showing, the bacterial targets included in the panel increasingly fail to reveal the etiology of a patient's disease. At the same time, nucleic acid amplification testing is known to fall short as a testing modality for the significant viral causes of ME.

In a point-counterpoint presentation at the Association for Molecular Pathology annual meeting last year, panelists debated this question: "Meningitis/Encephalitis Syndromic Testing in the Clinical Setting: Is It Ready for Prime Time?" Jennifer Dien Bard, PhD, D(ABMM), and Kevin Alby, PhD, D(ABMM), took on three topics: whether panel testing is appropriate when diagnosing ME, how serious the risks are of false-positives and false-negatives, and whether multiplexed panel testing for ME should be restricted.

The ME panel tests for six bacteria (*E. coli* K1, *H. influenzae, S. pneumoniae, L. monocytogenes, S. agalactiae*, and *N. meningitidis*), seven viruses (HSV-1 and -2, enterovirus, HHV-6, VZV, HPeV, and CMV), and the fungus *C. neoformans/gattii.* But the panel approach does not meet the current epidemiology of ME, so the chances of false-positives and false-negatives are significant, said Dr. Alby, currently associate director of clinical microbiology at the University of North Carolina.

Overall, bacterial meningitis rates have been dropping, and at least two of the panel's targets are falling off the grid as important causes of ME. "There's been a precipitous decline in both pneumococcal and meningococcal meningitis as the causative agent of bacterial meningitis in the U.S.," he said, crediting vaccination programs as the primary reason. In addition, two organisms or groups of organisms missing from the ME panel are significantly related to ME. "Gram-negative meningitis and staphylococcal meningitis are now equal to or eclipsing meningococcal meningitis" as causes of ME, said Dr. Alby, citing a chart of the incidence of bacterial meningitis by organism in the U.S. from 1997 to 2010 (Castelblanco RL, et al. *Lancet Infect Dis.* 2014;14[9]:813–819). "Many other bacteria are more likely to be causes of ME in many patient populations."

He noted that infections often stem from neuroinvasive procedures and are considered health care associated. Over an 11-year period at one hospital, there were about 80 bacterial infections and half were community onset, half health care associated (Srihawan C, et al. *J Am Geriatr Soc.* 2017;65[12]: 2646–2650). The pathogens linked to health-care–associated infections "are just as important but are not present, by and large, on the panel."

Obtaining a negative FilmArray ME panel, then, does not indicate that the patient can be taken off antibiotics because of the other potential bacterial pathogens. "Just because you have a negative ME test, you have not excluded infectious meningitis or encephalitis," he pointed out. In fact, in nearly 70 percent of cases of ME, whether acute or subacute, the causative agent of the diagnosis is unknown. Histoplasma, other fungi (native or iatrogenic), and other bacteria, especially with indwelling devices or zoonotic exposures, should also be considered.

Viral causes, in addition, are a significant cause of morbidity and mortality but cannot be diagnosed by nucleic acid methods, Dr. Alby said, citing arboviruses as a prominent example. In the Northeast, there was a particularly high rate of West Nile virus in 2016 (Burakoff A, et al. *MMWR Morb Mortal Wkly Rep.* 2018;67[1]:13–17). In

Pennsylvania, “through 2018 we’re already fourfold above our moving mean over the last five years.” If those patients come in with encephalitis, they may get tested and be found to be negative for a viral pathogen, then go undiagnosed. “They may not get that additional testing they need to diagnose their disease.”

So just using the ME panel with targeted organisms on it is not enough and even carries dangers, in his view. “It is not an exhaustive panel by any stretch of the imagination,” Dr. Alby said. “We still don’t know what’s going on because we don’t know a lot about this disease process.”

But Dr. Dien Bard, director of clinical microbiology and the virology laboratory at Children’s Hospital Los Angeles (CHLA), takes a different view based on her laboratory’s experience using FilmArray ME on 745 patients over more than two and a half years, with an overall positivity of 12.3 percent. “Panel testing is appropriate when diagnosing meningitis/encephalitis because it can rule out the majority of the pathogens of concern,” she said. Enterovirus is the No. 1 cause of meningitis that is identified, followed by bacterial meningitis, she noted. She believes a lot of the specific pathogens involved are included within the Film-Array ME panel. Adding herpesvirus as a cause “accounts for about 73 percent of all cases that you would see” (Hasbun R, et al. *Clin Infect Dis.* 2017;65[3]: 359–363).

That there are other significant causes of meningitis not included in the panel is not an argument against using the panel, she said. “If anything, the panel allows the provider to comfortably rule out meningitis caused by such pathogens as enterovirus or *Streptococcus pneumoniae*. The clinician can then investigate other etiologies based on the patient’s clinical presentation.” Enterovirus and bacterial meningitis are the top two causes of ME in pediatric patients, so the pathogens included in the panel are also highly relevant for children (Hasbun R, et al. *Ped Infect Dis J.* 2019;38[1]:37–41).

Contrary to Dr. Alby’s argument, she said, common pathogens in community-acquired CNS infections are included in the FilmArray ME panel. A study of 2,500 patients in 20 countries looked at community-acquired CNS infections and found “there were two top pathogens recovered: *S. pneumoniae,* which is part of the panel, and *M. tuberculosis.*” In addition, “within the groups of the elderly and the immunocompromised, Listeria, also in the panel, was a significant finding, along with VZV and *S. pneumoniae*.”

Dr. Dien Bard

Some targets are not included in the panel, she conceded. “But this is not a standalone test. It’s not going to provide you with all the answers. However, it would help for a great majority of the most common viruses and bacteria that you would want to rule out in the beginning of the patient workup.”

Before CHLA brought in the FilmArray, the majority of positives found were on target pathogens included in the panel, she said. “There are a variety of others that are identified, but this would of course require alternative samples, serological testing, etc.” Post implementation, with standard-of-care testing, all targets detected within the patient population of 135 were included in the panel. “In the patients that did not have a FilmArray ME panel, we had a total of three pathogens that were identified, two of which were targets included in the panel and an *Enterococcus faecium*, which is not included in the panel.”

Dr. Dien Bard believes that underuse of current diagnostic testing may be indicating misleadingly a decline in certain pathogens. “The decline may be due to the fact that the appropriate tests are just not being ordered,” she said. A study of test orders showed that enterovirus PCR was performed only for about 60 percent of all patients presenting with aseptic meningitis in adults, and for 74 percent of children. “For HSV PCR,” she said, “only about

39 percent of adults were tested, and only 16 percent of children." A great number of patients still did not have any sort of diagnosis (Shukla B, et al. *J Clin Virol.* 2017;94:110–114). Only 18.5 percent of the infectious etiologies were identified, and Dr. Dien Bard believes this may be due to test ordering patterns: "If you're not going to look for it, you're not going to find it."

A U.K. study published last year showed that prospective CSF testing on all of 638 patients showed a much higher positivity rate than is seen in other published reports; she believes that is "because they're testing it." With the targets that they performed on every single sample, "There are 29 cases of *Neisseria meningitidis* out of the 638, which is nothing to scoff at. The same with *S. pneumoniae.* The fact that they were able to identify these pathogens emphasizes the need for these types of broad-spectrum panels" (McGill F, et al. *Lancet Infect Dis.* 2018;18[9]:992–1003).

Standardizing ordering practices is necessary, Dr. Dien Bard said. "There's definitely no rhyme or reason in how certain tests are ordered or not ordered, and I think that's one of the main reasons why we're not confirming more cases." A study of the epidemiology of CNS infections in 358 children admitted to CHLA showed that etiology was identified in almost 60 percent of the patients. And, she noted, "all of the pathogens that were identified by standard-of-care methods, except for one *Staphylococcus aureus*, were included in the FilmArray ME panel" (Felsenstein, et al. Poster presentation at 25th ECCMID Congress, 2015).

For another patient at CHLA who had amyotrophic lymphocytic leukemia, the FilmArray ME identified CMV. The eventual diagnosis—meningoencephalitis as well as CMV retinitis—"was identified and treated appropriately purely because of this FilmArray result that we were able to provide them right away," Dr. Dien Bard said, showing the value of the FilmArray ME in detecting other significant pathogens associated with ME. "I do believe that a variety of these pathogens we've identified in our patients would not have been identified had we not performed the FilmArray ME, because even though we offered laboratory-developed tests for all the viral targets, the tests were rarely part of the initial sets of orderables."

"With these types of panels, you may actually increase the detection of bacteria that's not being recovered by culture. Inclusion of viral targets in the panel may also increase the chances of detection," she added.

In the decision to use or not use the FilmArray ME, should the risks of false-positives and false-negatives be a deal-breaker? That was the second question Dr. Alby and Dr. Dien Bard addressed.

The performance characteristics of the current ME panels, Dr. Alby said, increase the chance for patient harm. "Why do I say this? Well, there are high rates of false-positive bacterial detections that could lead to inappropriate antimicrobial therapy, false-positive viral detections can distract from other potential causes of infection, and then false-negative results, of course, can prolong diagnosis or inappropriately stop antimicrobial therapy."

A large multicenter evaluation of the ME panel showed that despite careful attention to standard of care and the discrepant analysis, 43 samples had results that were not present in the standard-of-care testing, a few falsely negative and a larger majority falsely positive (Leber AL, et al. *J Clin Microbiol.* 2016;54[9]:2251–2261). Extra detections by the FilmArray ME may be picking up more pathogens, he said. "But they are as likely to be a false-positive as to be a true positive."

Dr. Alby believes traditional testing works well. During his time at the Hospital of the University of Pennsylvania, he and colleagues evaluated previous positive CSF samples using the ME panel and confirmed five HSV-2, four enterovirus, and eight varicella-zoster virus. As expected, there were three additional targets detected—one HSV-2, one CMV, and one *H. influenzae*—that were negative upon repeat testing. The lab then tested 44 samples semi-prospectively. Most were negative for all targets, but two were positive for *Cryptococcus*, confirmed by culture; one was positive for enterovirus, confirmed by PCR. A sample that was positive for human herpesvirus 6 was confirmed by a secondary PCR test.

But false-positives posed problems. "The thing that held us back were the last two—one that was positive for *Haemophilus influenzae* and one that was positive for *Streptococcus pneumoniae*, neither confirmed by culture."

Repeat ME testing showed a negative result in both cases. “Then we looked at what was going on with the patients,” he said.

The patient with positive *H. influenzae* presented with altered mental status and left-sided weakness, and was discharged with a diagnosis of stroke, never given antimicrobial therapy, and recovered as expected for someone who had a stroke. The patient with false-positive *S. pneumoniae* had no pleocytosis and had a history of cutaneous T-cell lymphoma; that patient was discharged to hospice with no definitive diagnosis because other findings made clinicians think this was a transformation of the T-cell lymphoma.

False-negatives also carry dangers. A Mayo study of the ME panel on archived samples showed relatively good performance with strong positive agreement of the ME results with previously characterized samples for a number of targets. The ones raising concern were the HSV-1 target and the *Cryptococcus* target, Dr. Alby said. There was a particularly large number of false-negative *Cryptococcus* results. In one case, negative by the ME panel but positive by cryptococcal antigen and culture, “the patient was actually sent home, because they had a negative ME panel and when the bug started growing, they were called back to the hospital to be admitted and treated.”

In the second case, the clinician did not believe the result and requested a separate cryptococcal antigen test to be done concurrently with the ME panel; that test was positive. There was no change in therapy, however, because the physician didn’t believe the result initially (Liesman RM, et al. *J Clin Microbiol.* 2018;56[4]:e01927-17).

A 2017 case report showed that other patients may not be so lucky. The patient had a positive HSV-1 on an ME panel but showed no improvement with antimicrobial therapy. After being transferred to a different hospital where additional testing was performed, including PCR for tuberculosis, that testing was positive and the culture was positive for *M. tuberculosis* two weeks later (Gomez CA, et al. Open Forum Infectious Diseases, 2016).

The lesson: “Everyone was fixated on the positive HSV-1 as the cause of this patient’s illness and they stopped looking for anything else,” Dr. Alby said. “It wasn’t until the patient did not improve over the course of several days and was transferred to another hospital that the investigation continued. So this diagnosis was delayed because of the positive result.”

Dr. Dien Bard admits the ME panel has downsides. However, she argued that the benefits of the ME panel outweigh those limitations, and the cases Dr. Alby cited raise other issues worth noting. “I think the ME panel was a bit of a scapegoat in both cases,” she said. In the case of the false-negative HSV-1 patient, she contended that some fault may lay in the fact that the patient was presenting at a community hospital, where usually no infectious disease physicians are on staff and the care may not be at as high a level as at a tertiary care hospital—though, she noted, even at the tertiary care hospital, it took five days before ID was consulted and additional testing was ordered. “The patient was immunocompromised, moved from Vietnam 40 years back, and was at high risk for TB and *Crytoptoccus* infection. And these pathogens should have been on the differential diagnosis from the beginning.”

When she analyzed as many studies as she could find comparing the FilmArray HSV results against an alternative PCR assay, she found that for the most part “the results were pretty comparable.” Of almost 3,000 samples, there were nine false-negatives, one true positive, and three false-positives. “Most of the studies were just one or two off and often involved weak positives or preanalytical errors such as contamination,” she noted. And, she wondered, referring to the PCR test’s cycle threshold, “Is a Ct value of 45 positive really that significant clinically?”

The Mayo study finding of seven false-negatives was more significant. But Dr. Dien Bard wanted to highlight that discrepant results are assay- and site-specific. “It’s very dependent on what you’re using as a comparator method,” she said. “There are issues with the panel, but I think there is more to the story than just the fact that there was one test that made the biggest mistake—letting everything else off the hook.”

Her laboratory tested 32 samples with the exact same assay used in the Mayo study and picked up an additional HSV-positive on the ME panel, which was, after repeat testing on the direct alternative assay, found to be positive at a Ct of 39.8, a very, very weak positive, she said. “So at least in our institution we have not identified an issue with HSV sensitivity.”

Benefits like a significant decline in turnaround time—now at 2.4 hours at CHLA—outweigh the limitations of the ME panel, "which is why we're still using the tests," she said. One patient, a six-day-old, presented at an outside hospital where the HSV PCR was sent out on a CSF sample because it was not performed in-house. After the patient was transferred to CHLA two days later, the blood test was positive and the patient was treated with acyclovir, but died a week later. "The CSF result came back seven days later. I just don't think that's appropriate—seven days to wait for an HSV PCR result," Dr. Dien Bard said.

A multicenter retrospective study on neonates using the FilmArray ME looked at 16 patients who were positive for enterovirus by the ME panel as well as conventional methods to determine whether providing the result early on would have an impact on length of stay. Nineteen percent of the patients were discharged in 24 hours, likely due to the 17-hour TAT for conventional method enterovirus, and an additional 69 percent would discharge in less than 48 hours. But shortening length of stay to zero is even possible. "We speculate that if we're able to have a test that offers a rapid result like this and get a positive for enterovirus, you may not even have to admit the patient."

If laboratories are finding a lot of false-positives with the ME panel, "you should look at your process and see how you can fix it," Dr. Dien Bard advises. "It's important to remember that the ME panel is a PCR test and you're at risk for contamination like any other PCR test. The downfall of contamination is even more important because you're testing CSF. So it's extremely important to be aware of the risks."

"Generally, to deal with potential contaminants, we review the Gram stain, the CSF parameters, the clinical presentation, and patient history. Always communicate with the provider, and if possible get an ID consult. If we need to, we'll customize the reports to include comments." Repeat testing is a thing of the past in her laboratory. "We don't repeat-test any longer. If anything, we find it just adds to the confusion, where the result may be just a really weak positive or, due to some sampling variabilities, it's just not picked up the second time. So we've eliminated that."

She described the case of a child who was transferred from an outside hospital, together with a CSF sample, as an example of how the lab deals with false-positives and the importance of correlating with clinical findings and potentially other lab findings to avoid misdiagnosis. "It was a bloody CSF tap, so it wasn't great. *Streptococcus pneumoniae* was detected and the ID attending at the time and I agreed that this is likely not consistent with bacterial meningitis. We decided to report it anyway because they couldn't necessarily rule out ME, so we reported it with a cautionary comment that the sample could have been potentially contaminated." This case illustrates that contamination is a true concern, Dr. Dien Bard said, but can be prevented with proper measures.

Based on the wide range of pros and cons, Dr. Alby and Dr. Dien Bard last considered this: Should multiplex panel testing for meningitis/encephalitis be restricted?

"The ME panel opens the door for many laboratories that did not have the skill set or the infrastructure to perform these types of higher-level molecular testing," Dr. Alby said. "But the panel is not a test for all comers; it's not for all institutions." He believes CSF testing should be used only in limited circumstances with appropriate pre-test probability—that is, the probability of a patient having the target disorder—and ancillary testing services available. "And there needs to be some type of control over this test if it's going to be used."

A positive viral detection can be misleading, in part because some viruses are present in normal tissue, he noted. A patient who has a number of different primary CNS tumors, for example, may have HHV-6 in those tumors. The underlying concern are the tumors, not the potential presence of HHV-6 DNA in the CSF, he pointed out. "This is a good example of where detection of HHV-6 DNA in the CSF can lead someone down the wrong path."

The same thing could happen with patients in whom the panel detects HSV-1, HSV-2, VZV, or HSV-6. If these patients had some other cause or some other viral infection, given the biology of herpes viruses, that infection or inflammation can produce low-level reactivation of those viruses, Dr. Alby said. "The virus is there, the DNA is there, and if our assays are detecting it and we don't have appropriate controls and interpretations in place, clinicians will act on those results, even though they don't implicate the causative agent of the ME."

On the whole, meningitis/encephalitis is a rare event, Dr. Alby said. “Positivity in adults ranges from about one to two percent in terms of being able to diagnose the common meningitis viruses.” Seasonality is also a factor since time of year predicts positivity, as one study has shown with a chart of enterovirus results by date (Wilen CB, et al. *J Clin Microbiol.* 2015;53[3]:887–895).

Dr. Alby

So he believes in a more selective approach to ME testing. In the example reported in the same 2015 study, the institution set two criteria. “The patient had to have at least 10 white blood cells in their spinal fluid if they’re immunocompetent and then they could only do enterovirus testing if it was in the enterovirus season, which they classified as April through October.”

The effect of implementing this policy was that about a third of the tests for HSV-1/2, VZV, CMV, and enterovirus that were requested were not performed because they did not meet criteria. “This led to an annualized savings of more than $60,000 at that institution,” Dr. Alby said. An additional benefit, he noted, is that when fewer tests are performed where there is a low chance of positivity, “you won’t have to handle false-positive tests if you don’t perform the tests in the first place.”

Dr. Dien Bard, however, pointed out that meningitis can occur even in the absence of pleocytosis. She does not believe that multiplexed testing for ME should be restricted. “The approach at our institution is no restriction. Quite honestly, because the test was so new, we had no idea how to handle it, so we figured let’s just try it out and see what happens,” she said. “That being said, though, this is a test that we watch like a hawk, and it’s probably one of the few tests that we really communicate with physicians on.”

She cited two cases to illustrate why a lack of restriction was important. One was a six-week-old child with fever, irritability, and vomiting, with extremities poorly perfused, with tachycardia and tachypnea. “The CNS was completely benign and the white blood cell count was one. But this patient had enterovirus meningitis.”

A 34-day-old child with fever and fussiness also had completely normal CSF parameters, other than there being a lot of red blood cells in the CSF. This patient had parechovirus meningitis. As Dr. Dien Bard noted, a study of children under age 18 years demonstrated that in cases of bacterial meningitis, about 16 percent of the patients had a normal WBC count (Lin WL, et al. *J Microbiol Immunol Infect.* 2016;49[5]:723–728). In all of these cases, “if you were basing the decision to test solely on the presence of pleocytosis, the patient would not have been tested,” she said.

In fact, patients without CSF pleocytosis have a high rate of unfavorable outcome, another recent study showed. “In the adult population, particularly for HSV and meningoencephalitis, eight percent of the patients had an absence of pleocytosis, and the sequelae for them were high at 39 percent, with death for 21 percent” (Erdem H, et al. *Int J Infect Dis.* 2017;65:107–109). A CHLA study found, using the ME panel, that “Although pleocytosis was predictive of our ability to confirm bacterial meningitis in most cases, only about half of the patients who were positive for a viral target had any sort of pleocytosis.”

Restricting ME panel testing based on age, comorbidities, or other factors may make sense, Dr. Dien Bard noted. “I think that can be very institution-specific.” But she pointed to another reason why turning to a multiplexed panel for ME could appeal to institutions: cost-savings. A recent study developed an economic model to compare cost-effectiveness of three approaches—testing FilmArray ME on all CSF patients, performing standard-of-care testing, or FilmArray ME in the presence of pleocytosis (Duff S, et al. *Future Microbiol.* 2018;13[6]:617–629). “They found

the cost per patient was lower when FilmArray ME was performed on all pediatric patients presenting with ME."

Dr. Alby pointed out, on the other hand, that estimates of cost savings where more heavily multiplexed, more expensive tests are involved must take into account the cost of false-positives and treatment decisions that ensue from laboratory results. He cited a study that modeled the cost value of PCR screening for HSV, comparing the choice to screen all immunocompetent patients or only those with abnormal CSF findings. It found that increasing the significance of a positive test reduces the benefit of screening and the point at which pretest probability dictates screening varies depends on the cost of the PCR test (Hauser RG, et al. *J Clin Microbiol.* 2017;55[5]: 1566–1575).

As the point-counterpoint debate confirmed, critics and advocates of multiplexed panel testing for ME can agree that with ME patients, time to diagnosis is critical. But the best way to use panel testing to get to that diagnosis continues to involve tradeoffs that can be highly complex.

Anne Paxton is a writer and attorney in Seattle.