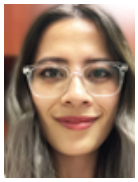
FilmArray ME panel—clinical trial to 1st clinical test

Anne Ford

May 2016—The BioFire FilmArray meningitis/encephalitis (ME) panel received FDA clearance last October, and in November Jennifer Dien Bard, PhD, D(ABMM), of Children's Hospital Los Angeles, presented the results of the multicenter clinical evaluation of the panel, in a webinar produced by CAP TODAY in collaboration with BioFire. The panel's use in the clinical setting will reduce turnaround time and may, pending further studies, have a positive impact on patient care and outcomes, said Dr. Dien Bard, director of the clinical microbiology and virology laboratories at Children's Hospital LA.



Dr. Dien-Bard

In February, she and colleagues got a further look at the possibilities when they ran their first clinical specimen using the ME panel on a neonate who had been seen initially at an outside hospital, where clinical presentation and abnormal findings from a cerebrospinal fluid specimen collected by lumbar puncture yielded convincing findings of bacterial meningitis.

"The patient's CSF specimen had markedly elevated leukocytosis, elevated protein, and low glucose levels. Unfortunately, bacterial cultures were negative and no organism was observed on Gram stain," Dr. Dien Bard told CAP TODAY. The patient was treated empirically for bacterial meningitis.

"When the patient was transferred to our hospital, we repeated the lumbar puncture, and all cultures were negative, despite consistently abnormal CSF chemistry findings. The patient was placed on broad-spectrum empiric coverage for bacterial meningitis, and because the mother's group B streptococcus screening was unknown at the time, group B streptococci was high on the list of bacterial etiologies."

At infectious disease rounds, Dr. Dien Bard heard the case being discussed and said, "Let's try running the sample and see what we can find." During rounds, she communicated with one of the virology laboratory's clinical laboratory scientists, who found the sample and tested it. Within a couple of hours, the result came back: The sample was positive for *Listeria monocytogenes*.

"Because of the finding, the clinician was able to switch to antibiotics that were more targeted for *L. monocytogenes*, and it was nice to have a definitive diagnosis instead of just treating for presumed bacterial meningitis with unknown etiology," she said. "And it came back in two hours from the time we discussed it. The fact that we were able to obtain a result so quickly, which then directly impacted the patient's therapy within a few hours from the discussion—it was exciting."

In the November webinar, Dr. Dien Bard, who is also an assistant professor of clinical pathology at the USC Keck School of Medicine, shared results from the clinical trials, including the panel's limitations.

The ME panel is a qualitative test that simultaneously detects six bacteria, seven viruses, and one yeast. In the performance study, which included analytical and clinical validation, limit of detection ranged from 100 to 1,000 CFU per mL for bacteria and yeast and 100 to 1,000 copies from overall viruses. Specificity studies among

more than 100 isolates at high concentrations revealed minor cross-reactivity of enterovirus versus rhinovirus, *Haemophilus influenzae with Haemophilus haemolyticus,* and *Cryptococcus neoformans/gattii* with *Cryptococcus amylolentus.*

"This should not really be too worrisome as the latter isolates are rarely isolated from CSF or from humans for the *Cryptococcus*," Dr. Dien Bard said. "Of note, the rhinovirus and the *Haemophilus haemolyticus* can be recovered from the respiratory tract, so avoidance of contamination is imperative. The ME panel was also found to be highly precise and reproducible at greater than 96 percent."

The 2014 clinical study included more than 1,560 prospective collected residual specimens from the 11 U.S. study sites. Just under 60 percent of the patients were hospitalized, with 34 percent from the emergency department. The comparator methods used were culture for all the bacteria, and PCR and sequencing for the viruses and yeast. The overall positivity rate was 8.7 percent, with co-infection occurring in five patients. Neonates showed the highest positivity rate (19.4 percent), while viral pathogens were the most common etiology, at 81 percent, followed by bacteria at 16 percent and yeast at three percent. For the most part, the sensitivity and specificity of each target was 99 percent or higher.

"Some targets were at lower performances, including the *Streptococcus agalactiae* where there was one false-positive and one false-negative; the enterovirus, which missed two cases of two positives; and HHV-6, which missed three positive cases and overcalled four cases," Dr. Dien Bard said. "The one false-negative case turned out to be a very low-level positive due to normal chemistry. The Gram stain was also negative with a presence of only one colony, so a very weak positive."

For *Streptococcus pneumoniae* there were 12 false-positives, five of which were confirmed by an alternative method or chart review or both, and the other seven were not determined.

There were seven false-negative cases of *Cryptococcus neoformans/gattii* determined by positive *Cryptococcus* antigen. "Interestingly, low levels of *Cryptococcus* antigen can persist for extended periods of time following therapy and resolution of infection," she said, "and when chart review was actually performed, it revealed that all seven patients were on antifungal therapy for *Cryptococcus* infection."

The ME panel has limitations, Dr. Dien Bard said. One of the most important: The sensitive nature of the test makes it especially important to guard against contamination—such as *S. pneumoniae* or *Haemophilus influenzae* shed from the respiratory tract of healthy individuals, or HSV-1 from people with active or recurrent cold sores—during collection and testing.

"Key limitations to communicate with clinicians include that HHV-6 and CMV can exist in latent form and can reactivate during infection caused by other pathogens," she said. "So infection with HHV-6 or CMV should be considered only when appropriate. In addition, VZV may not be the cause of CNS infection even when present due to the fact that if the zoster is suspected, viral shedding may occur in the CNS." And all results from the ME panel must be correlated with clinical findings, she stressed.

Dr. Dien Bard and colleagues examined the prevalence of meningitis and encephalitis in their pediatric patient population and what the utility of the ME panel would have been in a retrospective analysis. They looked at 358 CSF samples from patients seen in the ED. Of those, 8.9 percent were positive for a virus or bacteria. "When you add in all of the other positives from the variety of other specimens that were obtained from these patients, including blood, urine, stool, etc., a clinically plausible etiology, including both infectious and noninfectious causes, was identified in 117 of the 358, so a little less than 40 percent. Therefore, almost 60 percent of all cases did not have a definitive diagnosis."

Subsequently, Dr. Dien Bard and her team performed a prospective analysis of the FilmArray ME panel on 77 pediatric patients and compared the results to culture and molecular analysis. Thirteen targets from 11 patients were positive and eight targets from eight patients were confirmed by PCR and sequencing.

Three patients confirmed to be positive for enterovirus, parechovirus, and HHV-6, “interestingly enough, had no viral PCR studies ordered on them, not even HSV PCR,” she said. “Only bacterial cultures were ordered—again, highlighting the fact that with these conventional methods, you will only find what you think you are actually wanting to look for.”

To examine the potential impact that a rapid sample-to-answer panel could have on antibiotic exposure, Dr. Dien Bard’s team performed a retrospective analysis comparing an indirect HSV test offered only during first shift to a direct HSV method offered around the clock.

“We evaluated the impact that the faster test might have on patient exposure to acyclovir when a negative result is reported, since 99.9 percent of the time the result will be negative,” she said. First, the turnaround time decreased during this period, from 19.9 hours to 11.3 hours, “although for the vast majority of the time, the test is reported out within about four hours.” They then monitored the time from lumbar puncture to discontinuation of acyclovir and found that the mean time decreased from 29.5 hours to 15.5 hours. “Again, in the majority of these cases, acyclovir was actually discontinued within the one to two hours from reporting, which truly demonstrates that clinicians are directly reacting to the negative results or to the results in general that we are reporting out in the electronic medical record system.”
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