Sizing up ‘mega’ multiplex panels for respiratory viruses

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May 2013—During the flu season of 2012, patients crowded the emergency room at the University of North Carolina (UNC) Health Care’s Memorial Hospital. They presented with a cough. Congestion. Low-grade fever. In some cases, a sneeze. But in a matter of hours, their clinical pictures diverged: Some patients deteriorated, requiring hospitalization; others remained congested but stable.

Until fairly recently, the race to distinguish serious from benign pathogens depended almost exclusively on viral and bacterial culture. Despite the technological advances of recent years, viral culture continues to be the gold standard in terms of specificity, but it’s done at the often-prohibitive cost of time.

Amplification-based technologies for detecting multiple pathogens were born of the necessity for accuracy, speed, and a clear view of the pathologic big picture. To date, the FDA has cleared several multiplex panels for detecting multiple respiratory viruses, paving the way for clinicians to treat not just the single most obvious infection but sometimes co-infections that play a subtle—or not so subtle—role in the patient’s outcome. Of these panels, only a handful can simultaneously detect six or more respiratory viruses: Biofire FilmArray RP, Genmark eSensor RVP, Luminex xTAG RVPv1, and Luminex xTAG RVP FAST. These four represent the rise of the “mega” multiplex panel for respiratory pathogen detection, and a study published recently may well be the first to compare them to one another (Popowitch EB, et al. *J Clin Microbiol*. 2013;51(5):1528–1533).

The authors analyzed 300 patient specimens—including 200 retrospective and 100 consecutive nasopharyngeal swabs—using all four multiplex platforms. Their findings yielded individual profiles of the hands-on time, time-to-result, ease of use, and relative cost of each assay. The overall conclusion: Each assay has its tradeoffs.

Dr. Melissa Miller (right) with Elena Popowitch, MHS, medical laboratory specialist at UNC Health Care and coauthor of the multiplex study, at last month’s Clinical Virology Symposium. “Multiplex is absolutely a move in the right

direction," Dr. Miller says. [Photo: Julie Fletcher]

"My position has always been that there's a place for the majority of these assays," says senior author of the study Melissa Miller, PhD, of the Department of Pathology and Laboratory Medicine, UNC School of Medicine, and director of the molecular microbiology laboratory, UNC Hospitals. "It's a balancing act between workflow, expertise in the laboratory, the patient population you're serving, and the sensitivity you need to achieve."

At UNC's Memorial Hospital, where a large volume of patients flock to the institution's Center for Transplant Care, Dr. Miller notes a strong push from clinicians to detect adenovirus infections as reliably and efficiently as possible. Of the respiratory viruses that threaten transplant patients, the adenovirus is counted among the most severe. Early detection can alert clinicians to the need for additional blood or urine tests to prevent systemic infection.

For that reason, UNC's molecular microbiology lab uses the Genmark eSensor RVP, which relies on voltammetry to detect adenoviruses C and B/E, along with influenza A (H1/2009, H1, H3), influenza B, metapneumovirus (MPV), parainfluenza (PIV 1, 2, 3), respiratory syncytial virus (RSV A/B), and rhinovirus (RhV). While the Genmark panel works well for UNC's large transplant population, and returns results in 7.2 hours, the comparison by Dr. Miller and colleagues illustrates that the panel is time- and labor-intensive, and probably not necessary for every setting.

Of the four panels studied, the Genmark eSensor RVP is more or less matched in complexity by the Luminex xTAG RVPv1 and xTAG RVP FAST. While both of the Luminex systems rely on bead-based hybridization and detection, the RVP FAST delivers results in about 4.8 hours, about three hours before the RVPv1. "The RVP FAST attempted to lessen post-amplified manipulations, which I think we'll begin to see with other companies that have multiplex respiratory viral tests," Dr. Miller notes. "But because of that, they lost sensitivity to influenza A and B, among other viruses."

She and colleagues found that the eSensor RVP had an overall sensitivity of 98.3 percent and an overall specificity of 99.2 percent. The xTAG RVPv1 and RVP FAST had overall sensitivities of 92.7 percent and 84.4 percent, respectively, and overall specificities of 99.8 percent and 99.9 percent.

"The three more complex systems provide a high level of sensitivity at the cost of a complicated workflow and the need for molecular expertise," Dr. Miller says. "I've talked to other laboratories that don't have a large transplant population, so it's probably not as critical that they lose some adenovirus sensitivity. For their labs, it's more helpful to use a simple workflow that doesn't require as much molecular expertise."

On the other side of the country, Seattle Children's Hospital is home to one such lab. The core laboratory at Seattle Children's faced the onslaught of the 2012 flu season armed with a very different multiplex assay, the FilmArray, which detects 15 viral agents from respiratory samples in under 1.2 hours. An expanded FilmArray has since been cleared by the FDA to detect 17 viruses and three bacterial agents of respiratory infection: *Bordetella pertussis, Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*.

The FilmArray is simple and straightforward: An automated system extracts and reverse transcribes nucleic acid, then performs nested PCR in a single pouch. The closed system eliminates the risk of laboratory contamination. This feature also distinguishes FilmArray from most other multiplex assays, which require a technician to pipette post-amplified material. Cross-contamination during that pipetting step can significantly compromise a test's specificity.

Not surprisingly, the UNC study found that the FilmArray had an overall specificity of 100 percent. Though FilmArray offers slightly less sensitive detection of adenovirus and influenza B compared with the three other multiplex assays in the UNC study, lowering the overall sensitivity to 84.5 percent, FilmArray's streamlined procedure allows for a quick turnaround in urgent situations.

“FilmArray is one of the easiest and quickest multiplex panels to use,” says Xuan Qin, PhD, division chief of the microbiology laboratory at Seattle Children’s Hospital and author of a recent study in the *American Journal of Clinical Pathology* that describes the experience of implementing the FilmArray in the hospital’s core laboratory (Xu M, et al. 2013;139:118–123).

“The system is so well designed, it’s not actually what you think of when you picture multiplex,” Dr. Qin says. “This is a two-step PCR: It auto-extracts nucleic acid from the specimens, goes through the first step of multiplex PCR amplification, and then [the sample is] sent to a film array of 102 cells, each for a specific PCR.” To account for viral polymorphisms and increase sensitivity, each organism is covered by more than one target, and each set is triplicated. “It’s a very effective design,” Dr. Qin says. “When more than one target is positive for the same species, your sensitivity and specificity improve—the same principle behind our homebrew pertussis PCR.” (Qin X, et al. *J Clin Microbiol.* 2007;45:506–511).

Before implementing FilmArray, Seattle Children’s Hospital sent every respiratory specimen to a reference lab for direct fluorescence assay testing, a process requiring multiple steps—and bringing with it opportunities for error, Dr. Qin recalls. “It involved logging into our system and then transporting the samples to another lab and logging into their system. So there were two additional handoffs involved before we could report the results.” The entire process took about seven hours, at minimum.

After surveying 10 clinical laboratories across the United States and analyzing the daily workload at Seattle Children’s Hospital, the group invested in three FilmArray modules. The team opted to incorporate the system into the hospital’s core lab to ensure round-the-clock service for the hospital’s emergency department and urgent care center. It was a new experience for the lab’s 35 general medical technologists. “The core labs typically do CBCs and blood tests,” Dr. Qin says. “This was the first implementation of a diagnostic test for infectious disease.”

Though the technologists in the core lab had limited knowledge of microbiology, training was fairly straightforward: Faculty members, including Dr. Qin and study coauthor Min Xu, MD, PhD, program director of the core lab at Seattle Children’s, educated each shift of technologists about the basics of sterile technique, the importance of handling one specimen at a time under a biological safety hood, and practices specific to FilmArray such as the need to avoid bubbles when injecting patient samples into the reagent pouch. The instructors posted photographs on the board in front of the FilmArray analyzer to illustrate how the pouch should look if it is sufficiently filled versus insufficiently filled. An independent observer documented the technologists’ competency prior to the study.

“The core technologists were very happy to take on this responsibility. There were very few mistakes,” Dr. Qin says. The core lab typically transmits results electronically, providing limited interactions between the technologists who perform the tests and the clinicians who order them. But that has changed, at least for respiratory testing, with the introduction of FilmArray. “Now, the results become available while patients are waiting in urgent care,” Dr. Qin notes. “When [the technologists] get a positive for influenza, they enter it in and the feedback is, ‘Wow, this is great. We already know the result!’” Integration of FilmArray has made it easier for clinicians to prescribe antiviral drugs to the right patients within 48 hours of symptoms. “It has been a huge improvement in medical decisionmaking and patient management,” Dr. Qin says.

A minor drawback, Dr. Qin notes, is the lack of an interface between the FilmArray and the laboratory’s computerized data-entry system, which means that one technologist must enter the results and a second must review the information for accuracy. “Out of 4,000-plus specimens that we processed in the winter of 2011 through May or June of 2012, we had only one clerical mistake that was not picked up during the second review,” Dr. Qin notes. The mistake was quickly corrected and did not affect patient care. “Because this is such a significant clinical improvement, everybody has been very conscientious, establishing matrices for quality measurement,” she explains.

Another drawback of the FilmArray, described in the studies by Drs. Miller and Qin, is that the instrument, while fast, processes only one patient sample at a time. The tradeoff is that results for sick neonates or transplant patients can be ready in roughly an hour, but in an eight-hour day, each FilmArray can process only seven patient samples, while each of the other three multiplex panels can process 21 or more. Though most laboratories invest in multiple FilmArray systems—Dr. Miller has seen as many as 12 systems in labs with enough counter space to spare—larger laboratories that process 50 to 100 specimens a day would need an exorbitant number of FilmArray systems to keep up with the load. And every additional system adds to the cost: Labs that use three FilmArray systems—a throughput equivalent to individual Genmark or Luminex systems—spend three times as much as labs that use a single system. That's significant, considering the FilmArray reagents are already more expensive than those of the other multiplex panels Dr. Miller and colleagues tested.

But Dr. Miller is quick to caution that the FilmArray is not necessarily more expensive than the other assays in the grand scheme of things. "Though reagents are more expensive for the FilmArray, followed by the eSensor RVP, and the Luminex RVPv1, the hands-on time is reversed so it all averages out," she says. Each FilmArray assay requires five minutes of staff handling time—far less than the three competing multiplex systems—and can be performed by technicians who lack molecular training. The ensuing cost savings can be considerable. "The time that the technologist spends doing the testing, the time that a senior person spends reviewing the testing, the cost of overhead—all of these things factored into our cost analysis," Dr. Miller says.

Dr. Qin, too, acknowledges that the test isn't cheap, but cites significant savings on the clinical treatment side of the equation. "[With the FilmArray], there's less ED time: less contemplating whether to admit or not admit, to treat or not treat, to isolate or not isolate. It improves patient turnover. The cost of ED time is very expensive, so that gives significant savings."

With the expanded version of the FilmArray, clinicians and pathologists face an even greater dilemma: CPT coding. In the past, Dr. Qin points out, separate tests were used to detect each of the three bacterial pathogens that are now included in the expanded FilmArray, which was not available at the time of Dr. Qin's study. Though the viral and bacterial pathogens are now tested in a single reagent pouch, some clinicians provided feedback reflecting the desire and clinical confidence to opt to use a less expensive CPT code that covers only the cost of detecting the 17 viruses, or only the cost of detecting one of the three available bacterial pathogens. However, Dr. Qin calls the option of splitting a single test panel into several pathogen-specific tests "administratively difficult and financially irrational to payers." For example,"If we detect pertussis when it wasn't ordered, we have to call back to the clinician and explain that in order to report the pertussis results, they need to order the viral plus pertussis panel." To complicate matters, many of the pertussis-positive specimens often have viral co-infections. "This is an example of technology giving us enormous data that we don't know what to do with yet," Dr. Qin says. "The CPT coding modality has to be updated with the technology to make the cost manageable."

Cost savings aside, Drs. Qin and Miller acknowledge the benefits of having multiple types of systems in place—not just the FilmArray or the eSensor RVP, for example. Though batch systems are adequate for routine testing, they're far too slow when the situation is urgent. "If we've just started the [batch] run, it's going to be an additional 24 hours before that sample gets resulted," Dr. Miller theorizes. "We can get a faster result using singleplex or smaller multiplex assays, which have a quicker turnaround time, then get the full panel the next day."

Dr. Qin cites another reason to keep multiple tools in the toolbox: the constant threat of outbreaks. "When we moved to FilmArray, we determined that three analyzers would do the job based on the distribution of incoming specimens. But what if there's a huge outbreak? How would you handle the surge?" She has experienced this firsthand: Last spring and summer, a pertussis outbreak flooded the labs at Seattle Children's Hospital with more than 5,000 specimens. At the peak of the outbreak, Dr. Qin's lab received 160 specimens a day. "If you have three analyzers and the samples are coming out one piece at a time, you can't possibly analyze more than 23 specimens a day." How might a lab accommodate 160 specimens a day? The old standard, she says: batch testing. After experiencing the pertussis outbreak, Dr. Qin's group has calculated how many specimens they must process simultaneously to accommodate different levels of surge. They've learned to prepare for the worst but expect the best, particularly when it comes to the threat of an influenza pandemic.

“We have to think about surge and capacity, and keep some of the rapid influenza tests in the background just in case.” Faced with a surge, even DFA batch mode is not sufficient, Dr. Qin says. “You have to spin [the sample] down, you have to smear it onto the slide and stain it, you have to have a tech read the results. You can’t read 50 specimens at a time, so DFA is still not the modality for surge. You’d need a rapid test.” During a normal flu season, however, FilmArray more than accommodates the workload at Seattle Children’s. “We rarely have two specimens waiting in line, and even that delay is not significant compared with sending out DFA tests,” Dr. Qin notes.

Even though multiplex panels can detect multiple types of respiratory infections in a single shot, not all of the pathogens—MPV, PIV, and rhinovirus, for example—can be treated. But treatment is not the only goal of detection. “These results not only impact potential therapy for some of the viruses,” Dr. Miller says, “but also the ability to implement effective infection control practices for inpatients.” Premature infants, for example, can be given a monoclonal antibody against RSV to protect against infection. Many institutions rely on the findings to cohort patients based on their influenza subtypes: Clinicians wouldn’t necessarily cohort a patient who has MPV with a patient who has influenza, or a patient with influenza H1 versus H3.

Dr. Qin notes an additional benefit from the perspective of a children’s hospital: “For pediatrics, there’s a certain comfort in going home knowing that baby has rhinovirus and it’s going to run it’s course with just rehydration and Tylenol. It’s a positive way of antibiotic stewardship. Otherwise you might think, Oh, there’s a little redness in the ear or the throat. I’m going to prescribe amoxicillin just in case.

“There’s such an enormous benefit in reducing unnecessary antibiotics and also in isolating young patients to prevent transmission.”

The ability to type influenza strains was especially useful during outbreaks of the seasonal H1 influenza, Dr. Miller recalls. The seasonal H1 was resistant to Tamiflu while H3 was susceptible. “Being able to type these gave the physician information about whether or not they could use Tamiflu,” she says. The findings are also important for sentinel labs. “I feel like it’s a very important part of our job to be able to share with our public health colleagues that we’re seeing H3, we’re seeing H1, we’re seeing influenza B. Not every lab needs to be able to do that, but I think it’s important to have labs across the country with that capability.”

Are laboratories compromising sensitivity and specificity by moving to multiplex?

“When you look at what we’ve done for decades with viral culture, with rapid antigen tests, there’s no question we’re more sensitive now, even in the higher multiplex assays,” Dr. Miller says. In fact, Dr. Qin adds, FilmArray is so sensitive that two patients at Seattle Children’s tested positive for both influenza A and B after receiving FluMist three and seven days earlier, respectively.

That said, Dr. Miller notes, the specificity question is an important one. Because multiplex assays require pipetting a post-amplified material, even the most advanced technical laboratories face the risk of cross-contamination, which potentially compromises the specificity of some multiplex tests. The issue of specificity brings home the idea that every multiplex assay has its place, Dr. Miller notes. While the UNC study found that FilmArray upheld 100 percent specificity by performing the entire reaction in a closed pouch, which eliminates the possibility of contamination, the specificity of other assays depends on the aseptic technique of skilled molecular technologists. Dr. Miller cautions that labs should focus on what works best for their patient population: “It’s not worth doing a highly complex multiplex assay if you’re a small laboratory or one with no transplant patients. All of this factors in.” Still, she says, “Multiplex is absolutely a move in the right direction.”

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