Flu view—tests, predictions for the upcoming season

Ann Griswold, PhD

October 2014—Whether exotic influenza viruses will surface this winter remains to be seen, but one thing is clear: The coming season will pack a punch in terms of promising diagnostics and forecasting models.

Alere, Nanosphere, Cepheid, and Roche have new molecular tests for influenza that aim to transform the diagnostic landscape. And researchers are harnessing the power of big data to build stronger municipal- and national-level models of flu transmission that can potentially provide laboratories, hospitals, schools, and health officials with weeks of valuable prep time.

Against that backdrop of innovation, the Centers for Disease Control and Prevention anticipates that the nation may see a departure from the influenza A 2009 H1N1 virus that has prevailed in recent years.

"It's too early to tell, but just based on how homogeneous last season was with H1, it's likely that we might have a B or an H3 season," says Daniel B. Jernigan, MD, MPH, deputy director of the CDC's Influenza Division in the National Center for Immunization and Respiratory Diseases.

The CDC's prediction gives a nod to the finding that H1N1 has undergone minimal antigenic drift in the seasons since it emerged. "In looking at H1N1 using next-generation sequencing techniques, we can see that though the virus has undergone some genetic changes, it's antigenically pretty similar to the original virus back in 2009," Dr. Jernigan says. "It has been somewhat of a mystery that the H1N1 virus has not had any significant antigenic change yet. So at this point, we would think that the 2014–15 season likely will not be an H1 season."

The homogeneous H1N1 seasons of late have been noted with curiosity by many laboratorians, including Rodney C. Arcenas, PhD, D(ABMM), clinical scientist, microbiology/molecular, Pathology Consultants of South Broward, Memorial Healthcare System, Hollywood, Fla.

"In past years, we've seen maybe a handful of influenza A H3, and even less influenza A H1 wild-type strains. It's interesting how the 2009 H1N1 has now become the predominant influenza strain, at least in our area," Dr. Arcenas says.

Dr. Arcenas

During last year's season, Dr. Arcenas' laboratory began reporting separate trends for pediatric versus adult patients. The findings were surprising, he says. "What we found is that the adults seemed to have more incidence of influenza A than the pediatric patients, and of course it was predominantly H1N1, the 2009 strain," Dr. Arcenas says. Pediatric patients, on the other hand, presented with a more diverse mix of respiratory viruses—mainly rhinovirus—and more frequent coinfections.

"But as the season progressed, around November and December, we started seeing adults having more incidence of coinfection, primarily influenza A 2009 H1N1 with another virus, whereas kids, on the other hand, usually had rhinovirus plus something else," Dr. Arcenas says. "It's interesting how that shaped out over the course of last year's respiratory virus season, and I'm curious to see if that finding repeats this year."

The CDC's surveillance laboratories have picked up the low levels of flu activity that are typical in summer months, with some H1, H3, and B viruses, as well as two cases of the swine-associated H3N2 variant. "We don't know if those few viruses that we've seen so far are going to be the predominant ones that show up this fall," Dr. Jernigan says.

This year's flu vaccine is nearly identical to last year's, with the addition of a second B antigen. Whether that will provide sufficient coverage is unknown. "We are monitoring closely to see if the H3s are going to match what's in the vaccine," Dr. Jernigan says. "We're getting some hints that some H3s are not matching the vaccine components."

He reminds hospitals and laboratories that while H3 viruses tend to have more morbidity and mortality in the very young and very old, anything is possible. "It's hard to try to translate what happens at a population level to what might happen on an individual level, so flu should be kept in mind for all ages," Dr. Jernigan says.

As of September, there hadn't been indications that the flu season was about to begin. But if past trends continue, the CDC expects the first cases to begin trickling in earlier than usual.

"If you look at the last two seasons on the FluView website, the onset of the season was earlier than in past years," Dr. Jernigan notes. "Often you'll see the season start around November, gradually increase, and then peak sometime in February or sometimes even March. Last year it peaked in January. So a word to the laboratories is that, if this coming season is similar to the last two, then it's possible we might have an early season again, and it may be time to think about preparing."

Dr. Ledeboer

Being able to predict the start, peak, and intensity of a flu season is a long sought-after goal, but even the best forecasts often fall short. That may change, however, as researchers develop innovative methods of modeling infectious disease transmission.

"Flu tends to be one of those things that surprises us every time," says Nathan Ledeboer, PhD, D(ABMM), medical director of clinical microbiology, medical director of molecular diagnostics, and an associate professor of pathology at the Medical College of Wisconsin. "If you would have asked in January 2009 if we thought there was going to be a pandemic in the spring that ultimately went into 2010," he adds, "I don't think anybody would have predicted that."

The push to develop effective infectious disease models intensified last year, when the CDC offered $75,000 to the research group that could forecast most closely the number of flu-related outpatient visits during 2013–2014, in the agency's "Predict the Influenza Season Challenge."

The competition was a success, reports Matthew Biggerstaff, MPH, an epidemiologist with the CDC's Surveillance and Outbreak Response Team, Influenza Division.

"The methods and data sources that people used for this contest were things that we either weren't aware were being done, or actually hadn't been done until the contest," Biggerstaff says. From Wikipedia search terms to Twitter feeds, the 11 participating research teams explored unusual sources of digital surveillance data and novel

techniques to develop their models.

Watching the flu season unfold in real time held further excitement. “We definitely didn’t get 100 percent accuracy in the forecasts. But considering that this was the first year, we made good strides on the methods that are being developed,” Biggerstaff says. “The CDC hopes to continue this work on a voluntary basis with the group of researchers who participated in the challenge.”

The winning team, chosen for its method and accuracy, was led by Jeffrey Shaman, PhD, an associate professor in the Department of Environmental Health Sciences at the Mailman School of Public Health at Columbia University. For details on Dr. Shaman’s models, see “Predicting flu intensity.”

While hospitals await the first cases of seasonal influenza, diagnostics companies are gearing up. The need for reliable point-of-care testing, in particular, is driving the design of faster, simpler, more sensitive molecular diagnostics for influenza.

“Flu is one of those areas where delivering the results closest to the patients is going to be very, very important,” says Dr. Ledeboer, noting that no molecular diagnostics for influenza are approved as yet for point-of-care testing but that a few of the newer products are looking toward FDA approval.

For now, rapid antigen tests remain the mainstay for use in emergency rooms and physicians’ offices. The FDA proposal to reclassify them as class II devices, introduced last year, continues to move forward. The reclassification comment period ended Aug. 20; all comments are being incorporated now into the final rule that is expected to go through the FDA clearance process and take effect in May 2015.

Until then, the CDC hopes to foster greater appreciation for the art of specimen collection among nurses and clinicians. Two versions of the CDC’s course, Strategy for Improving Rapid Influenza Testing in Ambulatory Settings (SIRAS), for nurses and clinicians are available to the public on YouTube. So far, more than 4,000 people have completed the course, and 55,000 online visitors have viewed the SIRAS specimen collection videos, Dr. Jernigan reports.

“Relative to the actual number of people who do specimen collection, that’s not a bad number. I think it shows there are people out there who really would like to know how to collect specimens appropriately,” Dr. Jernigan says. “People are becoming aware that a positive test is a good thing and you can do something with that, but a negative test is something you don’t want to make a clinical decision on. You should really let your physical exam, your interview with the patient, and your overall assessment as a clinician guide you.”

At hospitals and clinics in the South Broward Memorial Healthcare System, rapid tests have their place.

“We provide the rapid influenza A and B antigen test as well as the rapid RSV antigen test,” Dr. Arcenas says. “We’ve been stressing to clinicians that a negative result doesn’t really rule out influenza A or B or RSV. But our pediatricians especially like that quick answer, because if it’s positive, that does help you.” If it’s negative, he adds, the pediatricians will sometimes then order the GenMark respiratory virus panel to get a more specific answer.

“Right now we still do the extraction up front, the PCR steps, the post-PCR cleanup steps, and then there’s a detection instrument. So it involves multiple steps and takes about six to eight hours, depending on the volume. But our clinicians really like the comprehensive nature of the panel. They’ve come now to rely on that full-blown panel, especially for our large population of complex patients,” he says.

GenMark is expected to seek FDA clearance for a next-generation, sample-to-answer instrument in 2015.

When it comes to point-of-care testing, Dr. Ledeboer argues, the tension between clinicians and laboratorians is palpable. “I’ve sat in meetings where clinicians have yelled at me and said, ‘I don’t understand why all of you laboratorians don’t understand the benefits of point of care.’ But I think that’s the absolute wrong assertion to make,” he says.

On the other hand, he says, if a practice makes sense and has an impact on care, it should be considered from a dispassionate, evidence-based perspective. “We shouldn’t fight technology,” Dr. Ledeboer says. “If a test ultimately delivers benefit, and if we can provide the right result in a point-of-care setting without putting a patient at risk, I’m 100 percent behind it.”

Molecular tests from companies such as Alere, Nanosphere, Cepheid, and BioFire suggest the possibility of point-of-care testing but do not have CLIA waivers from the FDA.

BioFire’s FilmArray Respiratory Panel, for example, tests for three bacteria and 17 respiratory viruses, including influenza A (H1, H1–2009, H3) and B, with an advertised turnaround time of about one hour.

Nanosphere’s Verigene Respiratory Virus Plus Nucleic Acid Test (RV+) detects seven respiratory viruses and subtypes, including influenza A (subtypes 2009 H1N1, H1, H3) and B, with an advertised run time of less than 2.5 hours.

Cepheid hopes to release a new molecular assay for influenza and RSV this season, if it wins FDA approval. Its current Xpert Flu test detects influenza A, influenza A 2009 H1N1, and influenza B viruses in about 1.25 hours. Last month, the company released its Xpert Flu/RSV XC molecular test outside the U.S. as a CE-IVD product.

Cepheid’s new Xpert Flu A/B/RSV XC test (XC stands for extended coverage) makes strides toward rapid result reporting with a newly launched feature called early assay termination, which alerts users to a positive result as soon as the cycle threshold value is achieved for detection of an individual target. Complete results are available in less than one hour but a positive result can be called in as little as 43 minutes, says David Persing, MD, PhD, Cepheid’s chief medical and technology officer.

“The new Flu A/B/RSV cartridge that we released ex-U.S. is a complete makeover. It is based on a comprehensive in silico analysis of influenza sequences going back to the pandemic flu strain that infected people in 1918,” Dr. Persing says. “Influenza viruses have a nasty habit of drifting genetically, sometimes in very inconvenient places within the viral genome, and that can affect assay performance.”

To circumvent the challenges of year-to-year and within-year strain variation, the company discovered new conserved regions within segments of the influenza genome that could potentially help compensate for variation in any one segment.

The ex-U.S. test includes five targets for influenza A (three seasonal, one for extended avian coverage, and one specific for H7N9), two targets for influenza B, and targets for RSV A and B. The U.S. version does not have an H7N9-specific callout but includes coverage for both seasonal and avian strains.

“We looked at influenza strain variation over the years for all the sequences that have been deposited. Any one single target has a 95 percent match with the sequence database, but that means we could be missing one in 20 cases. If, however, you combine three such targets, the predicted sensitivity approaches 100 percent,” Dr. Persing says.

“Interestingly, when we did the analysis against the 1918 pandemic flu strain,” he continues, “we found that one of the three targets in the flu A channel was mutated but the other two targets were a perfect match. So if that strain were to emerge again, we expect to be able to detect it based on the fact that there is a multiplicity of targets in the flu A channel.” Cepheid is currently pursuing CLIA waived status for its Flu A/B/RSV assay.

In other areas of the molecular market, Roche’s April acquisition of IQuum has spurred widespread anticipation of a CLIA waiver for the Laboratory-in-a-tube (Liat) Influenza A/B assay, which is designated as moderate complexity.

Roche intends to launch the system and assay by the end of the year, says Alan Garrett, director of strategic affairs.

“The system will enable non-specialized health care workers to perform rapid molecular testing in a point-of-care setting, closer to patients and with minimal training,” he says. “We have had 11-year-olds read the instructions, set

up the analyzer, run assays, and obtain lab-quality results."

The newly renamed Cobas Liat system, which has a turnaround time of 20 minutes for the Influenza A/B test, has 510(k) clearance and is a CE-marked system.

The recently launched Alere i Influenza A & B test is said to deliver molecular flu results in less than 15 minutes, thanks to a nicking enzyme amplification reaction, or NEAR, that proceeds at a constant 56°C and obviates the need for a thermal cycler. Targets are amplified within five to 10 minutes, and DNA purification is unnecessary.

Alere's new test straddles the line between the rapid and the molecular by combining a rapid turnaround time with the sensitivity of a molecular assay, says Norman Moore, PhD, Alere's director of scientific affairs for infectious diseases. The Alere i Influenza A & B test was launched in Europe in January, approved by the FDA in June, and released in the U.S. in September.

"I think it's fair to say that Alere's test is one of the revolutionary advances in molecular diagnosis because of the short turn time and the potential for point-of-care testing," says Yi-Wei Tang, MD, PhD, F(AAM), FIDSA, chief of the clinical microbiology service at Memorial Sloan Kettering Cancer Center and a professor of pathology and laboratory medicine at Weill Medical College of Cornell University. Dr. Tang is the senior author on one of the first clinical studies of the Alere i Influenza A & B test, published in the Journal of Clinical Microbiology (2014;52:3339–3344). The laboratory has been evaluating Alere's test for nearly two years on a research basis.

Limited hands-on time makes Alere's test ideal for use in the ER or in physicians' offices, Dr. Tang notes. "Other assays are very simple but you still need to pipette specimens. With this one, you don't pipette. It's considered a moderate-complexity test, but if I were the FDA, I would probably approve it as a CLIA waiver. Where's the complexity? You just put the swab into the liquid and that's it."

Issuing a CLIA waiver for a rapid molecular diagnostic like the Alere i Influenza A & B test could trigger an important shift in hospital epidemiology, Dr. Tang says. "This test would allow what I call real-time infection control. You try your best to discourage people from coming to the ER if they have flu, but you just cannot block them. But what if you can do a very quick test, get results within 15 minutes, and then rule out flu? If the patient needs to go to the floor, then they will be isolated. In addition, it's very important to pull the patients out of isolation if the test is negative."

Alere's Dr. Moore agrees. "In a lot of ways, the novel H1N1 crisis was a good learning experience for the entire country, because when novel H1N1 happened, people ran into the hospital, and that was the worst thing to happen. We got all these clinical samples, but there was no way for the state laboratories to turn them around and get the results back. So the idea here is that we potentially have a test that can hopefully be done on site in future epidemics, rather than having patients go to a hospital where they can infect other people."

That vision could soon become reality, Dr. Tang says. But he is careful to note that Alere's test isn't 100 percent perfect. "One very important con is that Alere's test has slightly lower sensitivity compared with monoplex PCR," Dr. Tang says.

In his laboratory's experience, the test offers a constant specificity of 100 percent, but sensitivity tends to vary according to sample type and flu type. The test has achieved a sensitivity of over 90 percent using fresh nasopharyngeal swab specimens, but only about 80 percent sensitivity when the swabs are placed in viral transport medium.

“Also, if we use the Alere assay for common circulating flu types like H1N1, H3N2, its sensitivity can almost reach 100 percent. For flu B, it’s great. But for unusual flu A types, there is relatively lower sensitivity,” Dr. Tang says. “So compared with rapid antigen tests, this is very useful and reliable. The rapid antigen test is not reliable in terms of sensitivity. But the price of Alere’s test is very high comparatively.”

His laboratory reported that the test, while roughly comparable in cost to other molecular tests for influenza, such as the Cepheid assay, is unlikely to be economical enough to replace rapid antigen testing in general hospitals.

Point of care is a pressing need, to be sure, but so is flexibility, says Adrienne Bambach, PhD, D(ABMM), Nanosphere’s manager of scientific affairs and acting director of clinical affairs. A newly expanded version of the Verigene—the Respiratory Pathogens Flex Test (RP Flex)—is available on a research-only basis as it awaits FDA approval. RP Flex will offer an expanded respiratory pathogen panel of 16 targets, among them four influenza targets and three Bordetella targets.

“What makes this test different is that we’re going to start offering a flexible panel with flexible pricing,” Dr. Bambach says. “We will manufacture the panel as a whole but customers will be able to select whatever targets are relevant to the physicians’ orders. What we’ve heard from our customers is that they want the ability to access a broad panel of analytes, but depending on how the flu season shapes up, they’d also like the ability to tailor their targets specifically to what’s happening in their area or their clinical setting, and to pay only for the targets they use.”

Dr. Ledeboer credits Nanosphere, BD, Alere, Cepheid, and BioFire, with their on-demand tests, “as having taken the ability to do flu testing and brought it to much more real time.”

“These tests have allowed us to make decisions about treatment and isolation much more effectively. The next step is, can we bring testing even closer to the patient?”

During the coming flu season, Dr. Ledeboer’s laboratory will initiate a study to explore whether molecular diagnostics placed near the point of care—in off-site clinics with laboratories, for example—can report results in a time frame that will stop the dispersal of unnecessary prescriptions.

The study will examine antiviral prescribing rates in three pairs of outpatient clinics in the Froedtert Health System, which includes three hospitals and more than 30 primary and specialty health centers and clinics throughout Wisconsin.

“We [at Froedtert] tend to deal with a lot of very, very sick patients. But by and large, the vast majority of our flu volume during season comes from our outpatient clinics and from our reference laboratory,” Dr. Ledeboer says. “We’re a fairly high-volume laboratory. During a busy flu year, on a busy flu day, we can do upward of 300 samples or more a day.”

Those who care for inpatients and ER patients will continue to rely on a sample-to-result test, so as not to interfere with the care of the critically ill.

The first pair of clinics in the study will continue to use the standard-of-care molecular test performed in the system’s central microbiology laboratory, with an average reporting time of eight hours.

The second pair of clinics currently use a rapid antigen test. “We all know the limitations of those antigen tests,” Dr. Ledeboer says. “So with that in mind, we’ll want to look at whether physicians are prescribing based on those results, are they not prescribing based on those results, and how often do they then have to go back and reexamine their diagnosis after sending for confirmation.”

The third pair of clinics to be included in the study have on-site labs staffed by medical technologists. Dr.

Ledeboer's group plans to introduce a near-point-of-care molecular test and inform physicians that results will be ready in 15 or 45 minutes, depending on the test used. The test will be selected after the group completes its validation of these tests.
After data are collected from all three groups during this flu season, Dr. Ledeboer's group will perform a retrospective analysis of outcome benefits. The analysis will determine how the health care system approaches flu testing in coming years.

As for this season, Dr. Ledeboer has a prediction of his own.

"A lot of it will depend on emerging or reemerging viruses, as we're seeing today with enterovirus D68. If we see a substantial amount of antigenic shift in a virus like H5N9 or any of those we've worried about in the past, then we might expect a big flu year. But I think a lot of that remains to be seen," Dr. Ledeboer says. "If you're asking me where my money is, I predict it's going to be an average flu year."

[hr]

Predicting flu intensity

Dr. Jeffrey Shaman's multi-model ensemble for flu forecasting was informed by data assimilation methods similar to those used in weather prediction. His models relied on recent flu activity as reported by three sources: real-time, municipal-scale data from Google Flu Trends; data from the CDC's Influenza-like Illness Surveillance Program; and regional- and national-scale influenza positivity rates from the CDC and World Health Organization. The team analyzed multiple ensemble forecasts each week to evaluate and adjust the model's reliability in real time.

Dr. Shaman

While the CDC's "Predict the Influenza Season Challenge" focused on predicting flu intensity at a national level, the Shaman laboratory has been modeling flu intensity at the municipal level for a few years. Flu predictions for 100 cities across the nation are published on the Shaman laboratory's website (http://cpid.iri.columbia.edu/), along with forecasts for other infectious diseases such as Ebola. In 2012, their most accurate year to date, the lab's models accurately predicted a peak in flu activity in 63 percent of the cities at least two weeks before the actual peaks occurred.

"We can also assign certainties to our forecasts. So we don't just say that flu is predicted to peak in five weeks in New York City; we can say there's a 70 percent chance that flu will peak in five weeks. And that's very different than if we said there's a 10 percent chance that flu will peak in five weeks," says Dr. Shaman, noting that the former prediction carries a much higher probability of accuracy because the ensemble of forecasts are in greater agreement.

Dr. Shaman cautions that infectious disease forecasting is just one tool in the public health arsenal. "It's not intended to do more than it is laid out to do, which is to provide a view of the influenza incidence that may be coming down the pike for various communities. It is used in complement with surveillance, and it relies on that surveillance. People have to start becoming comfortable with what it means, and how you might respond to a 70 percent chance of flu peaking in five weeks, versus a 10 percent chance of flu peaking in five weeks, versus a 70 percent chance of flu peaking in three weeks. Those are very different pieces of information." We have to learn how to use them, Dr. Shaman says, and how to incorporate them into decisions about public health responses and preparedness activities.

Forecasting efforts still have quite a way to go before the models are ready for mainstream use, says Dr. Daniel B. Jernigan of the CDC. “But as one of the participants in the forecast challenge said, 60 years ago weather forecasting was not very good either. And two things improved that. One was the technology and the analytics and all of the modeling and so forth. The second…was increasing the number of places that were actually collecting the data.”

In the meantime, flu forecasting models can enhance situational awareness.

“We currently put out information through FluView and other mechanisms,” Dr. Jernigan says. “There are apps you can download to tell you when things are trending upwards, and whether the circulating viruses are H3 or B, for example. We’re certainly continuing with this kind of information, but we’re seeing that we can actually improve our situational awareness through alternative data sources like Google and Wikipedia that can provide granular findings at the community level. That’s an area where we would like to continue working with our partners.”

As for Dr. Shaman’s predictions about the coming flu season, his influenza modeling efforts aren’t expected to kick into high gear until November. Until then, his team continues to explore multi-ensemble forecasting, including different model types and data assimilation methods, and different combinations of data that could enhance forecasts.

“We’re also looking at some other areas of the world, and forecasting, where some of the dynamics of flu may be a little bit different,” he says. –Ann Griswold

[hr]

Ann Griswold is a writer in San Francisco.