FDA, CDC, and tests steer flu Dx into new season

Anne Paxton

December 2013—What Soren Kierkegaard said about life applies just as well to flu seasons: They are understood backwards, but they have to be lived forwards. They're not easy to forecast. And perhaps that's one reason why the Centers for Disease Control and Prevention has just announced a "Predict the Influenza Season Challenge," offering $75,000 to the competitor who most successfully predicts the timing, peak, and intensity of the 2013–14 flu season using social media data.

It's certain that no two flu seasons are quite the same. Outside of the 2009 pandemic, last year's season was among the most severe in a decade, including the highest number of deaths of children from flu and the most hospitalizations in people over 65 since influenza became a nationally reportable illness in 2004. Nationally, the 2013–14 season has gotten off to a somewhat more typical start, says Daniel B. Jernigan, MD, MPH, deputy director of the CDC's Influenza Division. However, notes Rodney C. Arcenas, PhD, a clinical microbiologist with Molecular Pathology Consultants of South Broward Memorial Healthcare System, at his Florida facility the number of molecular respiratory virus panels performed in October was already matching the peak level of January–February from last year's flu season.

The volume of diagnostic testing for influenza overall, in fact, is showing a fairly consistent pattern. Since 2007, based on Medicare claims data, the number of rapid influenza diagnostic tests (RIDTs) as well as PCR-based flu and respiratory virus testing continues to grow even during relatively mild flu seasons, according to Julie Villanueva, MD, acting chief of the CDC virus surveillance and diagnosis branch. "While the amount of influenza-like illness is variable for any given season, when we look at the amount of diagnostic testing that's occurred, we can observe a substantial increase in recent years," Dr. Villanueva said in a presentation at the Food and Drug Administration last June.

With these trends, plus a pending proposal by the FDA to reclassify RIDTs as class II devices, and the introduction of faster molecular tests for flu viruses, Dr. Jernigan and other experts in influenza testing see new directions ahead for diagnostic testing of influenza.

The June FDA meeting convened an advisory panel of the Center for Devices and Radiological Health to discuss the agency's proposed reclassification of RIDTs in response to the poor performance of these tests during the 2009 flu pandemic. About 12 RIDTs are on the market today, with six of them CLIA-waived and widely used at the point of care. Under the proposed reclassification plan, which the advisory panel has approved, the FDA will continue to regulate the fluorescent antibody tests (DSFAs and DFAs) as class I devices, and influenza molecular tests as class II, adding an updated requirement for annual monitoring of molecular tests. But all current and new RIDTs would move from class I general controls to special controls.

If the proposal receives final approval, the RIDTs on the market would have one year from the final rule date to meet several new requirements, including new proposed sensitivity criteria, annual analytical reactivity testing with contemporary circulating influenza strains, testing of devices on newly emergent strains, and design controls. The FDA believes these changes will reduce the likelihood of false-negative results, help physicians diagnose patients accurately and treat appropriately, and enable effective infection control and public health response during influenza outbreaks.

The CDC has been working with the FDA to develop and implement the reclassification proposal for RIDTs, Dr. Jernigan says. At the June meeting of the advisory committee, "Basically there were four areas of decisionmaking around the use of objective criteria for evaluating these assays."

"First, when a device is being submitted to the FDA, should it have some standard sensitivity that it needs to achieve? Second, what is the comparison for the rapid tests—should it be culture, as it has been to now, or the

molecular assay, which has much higher sensitivity? Third, should there be an annual evaluation of the assay so we can see how they continue to perform against whatever circulating strains are prevalent? And fourth, should these devices be tested clinically if there is an emergence of a novel influenza pathogen as in 2009?"

Dr. Jernigan believes the FDA's proposed changes are significant. "They have the potential to really help move the landscape of these rapid tests at the point of care forward."

As to rapid antigen tests that are currently on the market, the CDC is pleased with some tests' addition of instrumented readers, which reduce the subjectivity involved in scoring visually read tests, Dr. Jernigan says. "We think that's very helpful in improving the performance of tests." However, he stresses that the agency is interested in seeing molecular influenza tests—currently only performed in the laboratory—at the point of care. "And it's my understanding that there's a lot of movement in that direction. We certainly look forward to having those assays FDA-cleared and especially if we can get them CLIA-waived. It's an area where there's a lot to learn, but the rapid tests at point of care still have a lot of issues of sensitivity that need to be addressed."

In 2010, the CDC initiated a strategy for improving the performance of the rapid antigen tests in conjunction with the FDA, the Joint Commission, the Association of Public Health Laboratories, and other partners. "We had three areas of focus," Dr. Jernigan says. "Better guidance, better practice, and better tests."

Working with the Joint Commission, the CDC developed a course called SIRAS—Strategies for Improving Rapid Influenza Testing in Ambulatory Settings. SIRAS explains the issues with some of the rapid tests but also goes through the algorithms for treatment—who should and should not get rapid testing. "So we've redone our entire testing and treatment guidance with the Academy of Pediatrics and the American Academy of Family Physicians, and it's now incorporated into a number of algorithms that different provider organizations use for their physicians," Dr. Jernigan says.

Included in that better guidance are iPad and iPhone apps that provide information about what influenza viruses are circulating in a particular region. "As a physician, if you know a flu virus is circulating in your community, the sensitivity of your own diagnosis increases. Your positive predictive value increases because of prevalence. So getting people this information is helpful," he says.

To improve practice, the CDC's SIRAS program also produced a series of nine YouTube videos on specimen collection techniques such as nasal aspirate, nasal swab, oral pharyngeal swab, and naso-pharyngeal swab. "Making sure clinicians know how to identify good healthy swabs can improve the performance of assays as well," Dr. Jernigan says.

Unfortunately, he says, the limitations of RIDTs have led some emergency departments and providers to stop using them. "What we're seeing in some places is they're not testing—but they're also not diagnosing and then they're not treating. There have been a number of studies, some of which we've done, looking at providers and antiviral usage and it's very clear many providers really would prefer to have the test. We know antiviral treatment generally increases when one uses the test."

As far as the current flu season is concerned, Dr. Jernigan regards the emergence of a new H7N9 flu virus in China last April as significant. "This virus did not cause a lot of disease at all in birds. But in humans who were infected, there was about a 35 to 40 percent case fatality rate. So this is very concerning because this virus may be able to move around the bird population very easily, and we only recognize it when it causes disease in people, and then the disease is severe."

Because of efforts in China such as closing of the live bird markets and improved surveillance, "we did not have any cases over the summer, which is usually when we don't see a lot of other avian flu either. But in early November, we've had four cases emerging. So we want to be sure we are monitoring this in case it suddenly takes off in China. There are no cases outside China or Taiwan so far, but there could be cases in Southeast Asia or

elsewhere. We just don't know."

There is a precedent for a flu virus with this high a mortality, he says: the H5N1 avian flu virus in 2004, which had even higher fatality rates. "These are the kinds of things we want to monitor closely to see if there's an emergence of potential pandemic strain, because the human population has no existing prior immunity to this virus. If it were to take off, it could travel very quickly around the globe. That's why we at CDC last April quickly developed an H7N9 PCR test that we shared with all the state public health labs, so they'll have the capability to diagnose H7N9 if they have a suspected case."

The CDC's ability to rapidly develop a molecular test allowed it to submit the PCR assay to the FDA for emergency use authorization, making it not a lab-developed test but an in vitro device cleared by the FDA as long as the potential emergency continues, Dr. Jernigan explains. Both the H5 assay and the seasonal assay have 510k FDA approval. "So CDC is essentially the manufacturer of these tests, but they make up only a tiny fraction of the testing out there, because under our approvals from the FDA, the tests are limited to specific high-complexity labs that participate with CDC in surveillance."

Dr. Landry

The clinical virology laboratory at Yale New Haven Hospital is one of the several dozen high-complexity laboratories that participate in surveillance with the CDC. Yale has been using the CDC-developed real-time TaqMan PCR protocol since it became available in early 2009, although the lab receives no reagents from the CDC. "That year, the seasonal H1N1 virus was resistant to Tamiflu," says laboratory director Marie-Louise Landry, MD, who is also vice chair of the Department of Laboratory Medicine at Yale. "The CDC PCR protocol included primers and probe sequences for both typing and subtyping assays, and we used subtyping to guide antiviral therapy."

When the 2009 pandemic H1N1 swine flu emerged and the primer and probe sequences were posted on the WHO Web site, the Yale laboratory had the primers and probes synthesized and set up the pandemic H1N1 subtype PCR immediately to be used for patients at Yale New Haven Hospital. "The CDC tests are very flexible, and CDC updates the primers and probes depending on what's in circulation," Dr. Landry says.

The CDC manufactures a 510k-cleared PCR assay, an in vitro diagnostic test that's limited by the FDA to use in public health labs. But because the implementation of the CDC protocol at Yale is a laboratory-developed test, the Yale lab has to purchase the components, prepare all its own reagents, and perform quality control on them, and that's a situation she would like to change. "We have in essence become the manufacturer. During flu season, it is very challenging to keep up with reagent preparation in addition to the testing." To make life easier, a couple of years ago Dr. Landry and colleagues reviewed the commercial molecular tests that were available. "One of the highly multiplexed tests we tested was not sufficiently sensitive. Then we tried a commercial real-time test. But compared to the CDC assay, the commercial assay detected 87 percent of the influenza As, 84 percent of influenza Bs, and 90 percent of RSVs. So again, it was not sensitive enough to replace the tests we were doing, and it was more expensive."

Another highly multiplexed test also looked promising because it takes only an hour. "But we found out the instrument took one sample at a time, and that wouldn't work with the workflow and volume we have. So we didn't go that route."

As a result, the laboratory continues to use its four ABI 7500 instruments with 96-well reaction plates, which allows it to conduct multiple tests for respiratory and other viruses with a fairly high throughput. During peak flu season, the laboratory runs a nine-target respiratory virus real-time PCR panel, which includes influenza A and B, two or three times a day. For public health purposes, influenza A subtyping by PCR is done once a week on hospitalized patients.

However, most labs are not in a position to run such extensive high-complexity testing and might benefit greatly from the new commercial molecular tests. “For laboratories that have been using rapid tests or cell culture, molecular tests offer many advantages,” Dr. Landry says, adding, “If I were in their position, I would probably be very pleased with the commercial tests that are becoming available.” Respiratory viruses take one to 10 days to become positive in culture, so multiplex PCR tests that are completed within one working day are a great improvement, she notes.

The Yale laboratory does offer RIDTs, but only on the night shift in the core laboratory when the virology lab is closed, and only for the emergency department to help with admissions, Dr. Landry says. The reason: “During peak flu season, hospital beds are in short supply, and we have many multi-bed rooms. So if a patient admitted from the ED is flu A positive, that patient can go into a multi-patient room with other flu A positive patients, if needed. However, if the patient is flu negative using a RIDT, the clinician knows that’s not a definitive answer.” During the day and evening shifts, the virology laboratory performs a multiplex DFA test with a two-hour turnaround time, and a respiratory virus PCR panel when the most sensitive result is needed.

“We had complaints from the ED that we did not have a RIDT available 24/7 with a 10- to 30-minute turnaround time. We showed them that the sensitivity was suboptimal, but when virology is closed, RIDT is currently the only option.” The RIDT result is considered a preliminary result and samples are retested in the morning by DFA or PCR.

In a newsletter that the virology laboratory sends to clinicians, Dr. Landry tries to provide guidance on the different test methods, and in the most recent issue, she indicated that the rapid flu tests may detect 20 to 50 percent of PCR positives, depending on the patient and the sample, while DFA will get 80 percent of PCR positives. “Clinicians should only obtain a laboratory diagnosis if results will change the care of the patient or influence the management of other patients, which is what the CDC recommends. So a test result can be useful for bed allocation, for better utilization of antibiotics, to diagnose lower respiratory disease, to allow earlier discharge, or to administer antivirals.”

Most hospitals without a virology lab, she suspects, would use a rapid test if they needed fast turnaround time. “But I think more hospitals are bringing in molecular tests, and there are a number of choices now. Several molecular tests require very little technical skill.”

She is particularly interested in seeing more studies that show the impact of influenza testing on outcomes and patient care. “What I think is missing is whether the additional money spent on testing really improves outcomes. Some papers on RIDT in children, for example, showed a reduction in antibiotic usage and ancillary testing. Other studies on immunofluorescence have also shown cost savings for all ages of hospitalized patients, including earlier discharge.”

By contrast, a Dutch study of molecular testing for respiratory viruses showed the testing increased costs and did not change therapy (Oosterheert JJ, et al. Clin Infect Dis. 2005;41 [10]:1438–1444). “In very ill patients or those who are hospitalized, we have a number of choices now, and I’d like to have more information on the cost-benefit ratio of the various test options. People postulate that molecular tests will save money and improve outcomes, but that remains to be proven. I suspect the answers will be different for inpatients versus outpatients, pediatric patients versus adults, complex disease versus uncomplicated infection.”

In terms of tests that have had an impact on care, Dr. Landry says, “The biggest change was when we started multiplex DFA in 2000. DFA using cytospin-prepared slides was as sensitive as culture and had a two-hour turnaround time when virology was open. Suddenly, we had a test that could affect clinical decisionmaking, and demand skyrocketed. But DFA is labor-intensive and highly variable between laboratories. With the 2009 flu

pandemic, PCR became the new gold standard. It is more sensitive than DFA, less operator-dependent, more suitable to high-volume testing, and can provide subtype, but the PCR panel also detects very low positives from resolving infections, multiple viruses in one sample, and a lot of rhinovirus, which people don't quite know what to do with. Sensitivity is a good thing, but it can have a downside because you may be detecting viruses that are not clinically relevant." Higher cost and a slower turnaround time compared with DFA, and the risk of cross-contamination and false-positives, are the main limitations now for PCR, she says. More studies on outcomes would help clarify optimal test usage and provide guidance on how positive results should affect clinical practice.

Dr. Arcenas

It's very useful for laboratories to establish benchmarks and metrics on their influenza testing, Dr. Arcenas says. When his laboratory in Hollywood, Fla., first brought in molecular testing for respiratory viruses, "We put out to our clinicians for a couple of years a viral prevalence report to give them an idea of what's circulating in the patient community and coming into our hospitals. Then it really dawned on us, since we serve pediatrics as well as the adult side, to compare data on them in a virogram. And we found some interesting things. This year there is an earlier occurrence of influenza A in adults where it seemed to be lagging in pediatrics." Now, pediatric numbers are creeping up, Dr. Arcenas says, "and we're also seeing an earlier incidence of RSV and higher incidence of co-infections in pediatrics and adults."

He and colleagues published an article on inappropriate and obsolete clinical laboratory tests, which focused on rapid antigens and how they're suboptimal in performance (Kiechle FL, et al. Clin Chim Acta. 2014 [Jan. 1];427:131–136). "I guess it's hard for pediatric clinicians to get rid of the rapid tests. In their defense, a positive result does help them out and for molecular they have to wait at least a day. But negative results don't help at all. And that's kind of an issue here."

On the adult side, clinicians tend not to order a lot of rapid antigen tests, he says. "If the patient has the flu, they go ahead and treat it, and they get the extensive molecular panel if they're going to admit the patient or if they have someone who is immunocompromised."

Dr. Arcenas expects to see more tests emerge soon to occupy the middle ground between rapid antigen tests and the full-blown molecular panels. "Some companies are already offering FDA-approved 'direct-to-answer' molecular tests, where you can actually test the sample. You don't have to do any kind of nucleic acid extraction or set up a PCR master mix," he says. "You just put in the sample, it goes through the reverse transcriptase PCR reaction, then detection, and you get a result in one or two hours. It primarily targets more common viruses like influenza A and B, and RSV, which are the ones that really have treatment implications where a rapid test matters."

The FDA is proposing to reclassify waived rapid tests, he adds, because of the poor performance of the tests. "Of course, you have to really monitor the tests each year because the virus does change, and class II classification should be a way to have these companies that make the RIDT be more vigilant in making sure their assay is performing up to their claims."

In the meantime, Dr. Arcenas continues working at his hospital to get clinicians to understand the limitations of the rapid tests. "We had a lot of success during the 2009 H1N1 outbreak when all those papers came out on poor performance. So we had many clinicians treating symptoms as flu and ordering the full-blown panel we were

offering if warranted. For some of our high-risk patients on the pediatric side, I think just because of the nature of the disease state, they will go ahead and get the whole panel to cover. So they're slowly switching, but the need for the panels is still not completely recognized."

Despite the emergence of rapid molecular assays, the cost of molecular testing for influenza will need to drop for molecular tests to compete with RIDTs, the CDC's Dr. Jernigan says. "As these molecular assays become more automated or simplified, the price point has to be lower. But overall, over the next year we look forward to seeing some promising new technologies coming out that we think will improve the diagnosis of influenza."[hr]

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