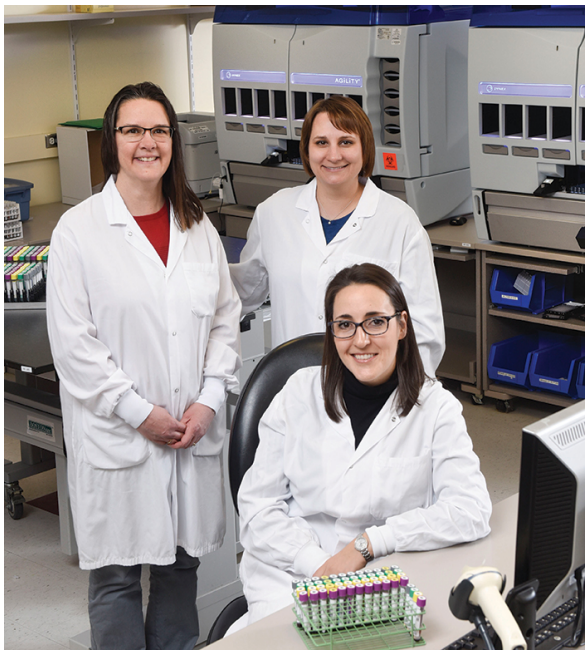
TB testing: new approaches to old scourge

Karen Titus

April 2018—Scratch the surface of TB testing, and things quickly get interesting.

The standard skin reaction test, widely adopted by the early 1940s, is still in use today. The goal has remained steady as well: break the transmission cycle. “From the clinician perspective and the laboratory perspective, because of its infectious nature, we want to identify people with latent tuberculosis,” says Elitza Theel, PhD, lab director for the infectious disease serology laboratory, Mayo Clinic and Mayo Medical Laboratories. “The ultimate goal is to treat them, so they don’t progress to active TB.”



Mayo Clinic began offering the QuantiFeron-TB Gold Plus in February, a switch that required logistical juggling, say Dr. Elitza Theel (seated), Lori Misner (left), and Heather Hilgart. “Pay attention to all impacted areas,” including phlebotomy, Dr. Theel advises other labs.

But latent TB by definition, of course, cannot be detected directly—assays are based on detecting the cell-mediated immune response to *Mycobacterium tuberculosis*. The fact that the skin test is still in wide use is perhaps indicative of how pernicious TB is, and how difficult it can be to develop and adopt new TB tests. Blood-based interferon-gamma release assays, or IGRAs, arrived on the scene just in the last decade or so. Tests from two companies have FDA approval: the T-Spot.TB (Oxford Immunotec) and the QuantiFeron-TB Gold Plus (Qiagen). The latter test will replace the third-generation Gold test, which the company says it plans to discontinue on June 30.

If Dr. Seuss were weighing in at this point, the tale might read something like this:

Old test, One test, Two test, New test!

But the story is far from over, given the estimated 13 million residents in the U.S. with latent TB infection. Among those, about five to 10 percent will develop TB if they don't receive treatment. So what lies ahead?

Several changes are in the works.

One was a recently updated treatment guideline from the American Thoracic Society, Infectious Diseases Society of America, and Centers for Disease Control and Prevention that calls for stratifying patients into three groups based on risk of progression: high risk, low to intermediate risk, and unlikely to progress or to have TB.

A second is Qiagen's move to a fourth-generation assay. Like other earlier IGRAs, the Plus test (as it's generally known) detects CD4 T cell response. But the newer test also detects CD8 T cell response, an addition many hope will prove beneficial on several fronts.

Finally, the CDC in February sent out a notice that it plans to change medical screening guidelines for immigrants, effective Oct. 1, calling for the use of an FDA-approved IGRA for TB screening, including children under age five.

All these moves will likely nudge laboratories in new directions.

Even before the CDC announcement, some health care providers had begun to move away from the skin test, says Dr. Theel, who is also co-director of vector-borne diseases laboratory services and associate professor of laboratory medicine and pathology, Mayo Clinic. Specificity can be a problem with the skin test, she notes, and it can be falsely positive in individuals who have undergone BCG vaccination or if the test was administered or interpreted incorrectly. And sensitivity, particularly in patients who are immunosuppressed or in very young children, can be lower.

For annual health care employee screening, many institutions are making the switch from skin testing to an IGRA as well, says Dr. Theel, who reports seeing this among Mayo's reference lab clients. Collectively, she says, referring to Mayo's reference lab testing and its own patient population, which includes a large number of patients from TB endemic areas, "we performed over 90,0000 QuantiFeron tests last year."

About an hour and a half up the road, in Minneapolis, Glen Hansen, PhD, D(ABMM), is also wrestling with a diverse patient population at risk for TB. As the medical director of clinical microbiology and molecular diagnostics at Hennepin County Medical Center, he oversees a high volume of TB testing. "We have a big refugee screening program here, and the hospital supports a public health TB clinic."

As for employee health care screening, he says, the first question to ask is what percentage of the institution's workers are foreign born. "Our hospital," he notes, "continues to have and staff a fairly diverse group of people." Many of these people are unlikely candidates for the skin test because they've received the BCG vaccine.

While some institutions are deciding to move to less-frequent worker screenings, he adds, that's not the case at Hennepin. "We continue to see a fair amount of active TB cases at our hospital. So yearly screening is still something we feel strongly about."

While the winds appear to be shifting in favor of IGRAs and, in the case of those who use the QuantiFeron assay, toward the Plus test, diagnostic complexities remain. Michael Wilson, MD, puts it this way: "It's critical that you understand the strengths and weaknesses of the test you're using because there aren't a lot of options."

Dr. Wilson is well versed in both skin testing as well as IGRAs. "We currently use the QuantiFeron Gold assay and are moving soon to the Plus assay for high-risk patient populations," says Dr. Wilson, director, Department of Pathology and Laboratory Services, Denver Health Medical Center. "But we use skin tests for low-risk populations. Which test to use depends on the patient population." The skin test is used for annual and new employee screening and other populations at low risk for TB.

Dr. Wilson

One benefit of the skin test, he says, is that it's quite a bit cheaper than an IGRA, at least from an organizational standpoint. "I know not everyone agrees with that. But it is less expensive if you're testing large numbers of people," says Dr. Wilson, who is also a professor of pathology, University of Colorado School of Medicine, Aurora. Labor costs for skin testing are low because it takes only seconds to apply and read the test, he says. There's no need for phlebotomy supplies to collect blood, or for automated equipment or verification. And the reagents and supplies are inexpensive.

Yet skin testing has many flaws, says Dr. Wilson, echoing the aforementioned reasons. "We all know it can be difficult to interpret," he adds. In addition, simply making the interpretation can be a logistical hurdle, since it requires a follow-up visit within 48 to 72 hours.

On the plus side, he says, are the decades of experience behind its use. From this perspective, the minuses may not be so much of a minus; rather, it's a plus to already know what the limitations are. "We know very well when it works and when it doesn't," he says. Assuming there's no such thing as a perfect road, maybe it's enough to know where the largest potholes lie.

Dr. Wilson says he appreciates the advantages of IGRAs as well, including the need for only one patient visit, more-objective test results, and the fact that prior BCG vaccination does not cause false-positive test results.

But he frets about the occasional peculiar result. "It's a small number," says Dr. Wilson, "but there are patients who have very odd reactions where some of the controls don't work. So you have these rare test results that are not well understood." In addition, in one study, a low but not trivial number of patients at low risk for TB had indeterminate test results, he says, which led to additional follow-up testing. "Whether the new generation of IGRA tests will have similar issues has yet to be determined," he says.

Given that Dr. Wilson finds the 20th-century roots of the skin test reassuring, it's not surprising that IGRAs can feel youthful in comparison. There are still questions he'd like to see answered by clinical studies. The CDC's call to use IGRAs to screen immigrant children, he says, could be a blinking caution light, given that the tests are not FDA cleared for children under age two. "There's no reason to believe it doesn't work well," he says, "but there is that gap."

No matter how diligent and careful manufacturers may be, he adds, "There are always surprises. When you use it on a large scale, things always come up."

Other gaps exist over the potential value of detecting CD8 T cell response.

Though Mayo has already switched from the QuantiFeron Gold test to the Plus test, Dr. Theel says she'd like to see more peer-reviewed literature showing the advantage of using the newer assay. The addition of CD8 positive T cell stimulation offers the potential for increased sensitivity in the case of active TB, she says, but her own literature search hasn't yielded enough data to convince her yet. Ditto for studies that show the Plus test is more sensitive in HIV-positive patients with low CD4 T cell counts. "I think the potential is there," she says.

Dr. Hansen, whose laboratory has also made the switch, suspects measuring CD8 response will be helpful, although he notes the data to date are mixed. In his own experience, the new test shows a bump of four to eight percent increased sensitivity—an improvement he says has also been well established in the literature. "We're also seeing improved performance in patients whom we struggle with," particularly those with HIV.

“Down the line, we should have the benefit, hopefully, of risk stratifying patients in ways we’ve been unable to do before,” Dr. Hansen says. Mounting both a CD4 and a CD8 response, or a CD8 response alone, could identify people who may be at higher risk to progress to active TB. “We know CD8 response is a marker of burden of how the host-immune system responds to an intercellular pathogen.” It’s also known that decline in CD8 response is a marker of whether treatment is working, he says.

In short, he adds, “I’m optimistic that, in time, the stratification with CD8 response will turn out to be clinically useful.”

Despite his optimism, he’s quick to acknowledge the test “is not magic. I tell that to people all the time.”

Dr. Hansen

Nor are IGRAs easy to run, says Dr. Hansen, since they involve some manual processing. He says he makes a point of reminding lab colleagues of the test’s implications, both in terms of public health and committing patients to therapy. Treatment is not for the faint of heart—duration can last up to nine months, with the range being three to nine months depending on the medication with daily, weekly, or twice weekly dosing intervals. And it can be toxic.

“So the test needs to be handled with a degree of respect,” Dr. Hansen says. This includes setting up protocols that allow laboratory professionals to dedicate themselves to running the test, “so they’re not asked, for example, to process this test and then baby-sit three instruments across the hall.”

At his laboratory, Dr. Wilson says, there’s one full-time professional who handles only IGRA TB testing. “That’s all he does.” The need for such expertise is obvious to Dr. Wilson. “It’s a subtle test,” he says. “It’s not a straightforward, black and white test at all.”

Making the switch to the Plus test can also be challenging, say those who’ve done it. One of the most obvious changes is that the new test involves an extra tube.

The Gold assay uses three tubes to collect whole blood. One, the nil tube, basically functions as a negative control that looks for circulating levels of interferon gamma, Dr. Theel says. The second is a mitogen (or positive) control tube, used to confirm that a patient has active lymphocytes that can be stimulated to produce interferon gamma. And the third, a TB antigen tube, contains peptides (for ESAT-6, CFP-10, and TB7.7) designed to stimulate primarily CD4 positive T cells.

The Plus assay allows for collection of whole blood in a single lithium heparin tube, which can then be sent to the lab and aliquoted into the four Plus-specific tubes. Dr. Theel calls this an “improvement for phlebotomists and patients,” although it adds to the laboratory’s workload, she says, referring to aliquoting, and increases the likelihood of mislabeled specimens in labs that perform a high volume of QuantiFeron testing. Processing is essentially the same.

The Plus also has nil and mitogen control tubes; unlike the Gold assay, it has two TB antigen tubes. TB1 is designed to stimulate only CD4 positive T cells, similar to the Gold test, although the TB antigens differ—in the newer test, TB7.7 has been removed, while ESAT-6 and CFP-10 remain. The TB2 antigen tube contains both traditional peptides and additional, smaller ones that have been genetically engineered to stimulate a CD8 response.

Mayo began offering the Plus test on Feb. 13. For Lori Misner, CLS, and Heather Hilgart, BS, two technical

specialists at Mayo, the switch created a few headaches. Single-tube collection might be helpful for smaller labs and clinics that don't have the ability or resources to perform incubation and centrifugation, they acknowledge. But Mayo, a large international reference laboratory, requires clients to send the completely processed tubes containing plasma ready to be placed onto an automated instrument. "We do not accept the single lithium heparin tube with whole blood," Hilgart says.

The verification process was also a challenge. "At least in my opinion," says Dr. Theel, citing two factors. "One, you need fresh whole blood, so you essentially have to enroll individuals. And you need patients who have latent TB, so you need to have a good positive and negative pool." The lab enrolled low-risk, skin-test-negative Mayo employees for the negative controls, but had to look elsewhere for positive samples.

"We started by enrolling skin-test-reactive employees. However, they didn't always yield a positive QuantiFeron result," Hilgart says. "So we had to expand our search, and we collaborated with our local TB clinic" to obtain positive QuantiFeron specimens.

The switch involved some logistical juggling, says Misner, since the lab also builds QuantiFeron collection tubes as kits for its clients. Adding the fourth tube meant giving a 60-day notice about the switch and required numerous conversations with clients about using up older kits in a timely fashion. "That was tricky," she recalls. "How do you get clients to stop drawing the three tubes and start sending the four tubes?" Overall the switch went fairly well, she says. "But you still have those people who are ordering the correct test but using the wrong tubes."

Hilgart sounds somewhat amused when she recalls the whole process. When they first learned of the impending change, she says, it sounded simple: *We're just adding this extra tube.* She laughs. "Given the reality of lab workflows, it's more like, *Oh my gosh, they're bringing in an extra tube!* It really did change things for us. We went from running 25 patients at a time to 21 patients at a time. It affects your LIS. And it affects our postanalytic—when we're done with the tubes, we have to make more room to store them until we're able to dispose of them. We're using more reagents, more plates, each day now, and we have to make more space in the refrigerators to do all that. So there were a lot of workflow changes we had to make just for our one extra tube."

Not that either is complaining about the potential usefulness of the test. "It can be beneficial," says Hilgart. "But there's a lot of work involved in making things better for patients."

Dr. Theel says, "We did do a lot of education for internal and external providers." That communication was critical in making the switch, she continues. "Pay attention to all impacted areas," she advises, including phlebotomy.

Giving that heads-up may be one reason she's received no calls from clinicians questioning the additional tube result since the laboratory switched to the new test. That silence doesn't necessarily tell her what their experience has been, she says. "On the other hand, it could mean that nothing significant has changed from their perspective or that the interpretive comments on the reports are sufficiently informative."
Hennepin County Medical Center switched from skin testing to IGRAs about a decade ago. Dr. Hansen has used both the T-Spot and the QuantiFeron assays. "They're both great tests," he says. In November 2017 the laboratory went live with Qiagen's Plus test.

Dr. Hansen is enthusiastic about the new test, though he says it hasn't necessarily rubbed off on his clinical colleagues. "We're not getting a tremendous number of calls from physicians asking for a fourth-generation test," he says with a laugh, before adding, "I do think we'll get there."

He does handle numerous calls from physicians in response to a positive test result in a patient with appropriate risk factors. "It probably necessitates a discussion with that patient about whether they're committed to treatment," he says.

The most frequent call he gets concerns low-risk patients who test positive. In those cases, he says, he queries his clinical colleagues closely on possible risk factors that might be present. If none emerge, "I tell them it's a false-positive result." While rescreening the patient is an option, he reminds them of the importance of risk factors.

“Why are they ordering the test in the first place? Everything defers back to what we know about the patient, and their history, and potential TB exposure.”

“We’ve had a couple of patients,” Dr. Hansen says, “who had been given the influenza vaccine right before they were given the [TB] test.” The results were borderline positive, likely due to the release of nonspecific interferon, stimulated by the vaccine. “The treatment decision belongs 100 percent to the treating physician. But we give them support” and remind them that false-positives can be expected with an immunological test.

The aforementioned ATS/IDSA/CDC updated treatment guideline makes special note of patients who are unlikely to progress or to have TB. For this group, says Dr. Theel, “They flat out state that testing is not recommended. But if it is performed, they recommend to start with an IGRA.” A positive result should then be followed by a second test, either an IGRA or a skin test. “Only if both tests are positive should the patient be considered a positive.” The reason for the dual testing is simple, says Dr. Theel: “They’re aware of the false-positive results in low-risk patients. And this is just one way to help provide some guidance on how to handle those with positive results.”

“The most frequent call I get,” she continues, “is for these low- to no-risk patients who have a very low positive result, like 0.5 IU/mL. And the question is, ‘What do I do with them now?’” (The cutoff for negative is < 0.35 IU/mL.)

Dr. Hansen urges caution as well, noting that interferon gamma is not unique to tuberculosis. “So we defer to clinical consultation for appropriate risk factors.”

Risk factors can be difficult to ferret out, Dr. Wilson says. “We’ve learned that the hard way.” Electronic health records don’t necessarily make the task easier. Although they have fields that ask about travel outside the United States, the answers can be too simplistic to be useful, he says. Take someone who reports having recently traveled to South Africa, for example. “Did you work in a health clinic for a month?” he asks. “Or did you go to a two-day meeting in Cape Town?”

Moreover, domestic exposure can be easily overlooked, he says, recounting the recent case of a local family with a visiting uncle who had the disease. “TB control experts are used to probing these matters,” Dr. Wilson says. Not every institution is so well staffed, however.

The search for a better TB test is, like a Twitter thread, seemingly endless. “Many of the studies that have looked at how well tests perform in latent TB populations do so by using whether or not the patient grew an organism,” Dr. Hansen says. “But that’s not a great gold standard because, by the definition of latent TB, it’s hard to get an organism. So we’re looking for tests that always push the envelope of increased sensitivity and specificity.”

Physicians would love a rapid test that tells them if TB is present, says Dr. Wilson. They’d love to have molecular susceptibility testing immediately available.

And why not throw in another possibility: How about identifying those who are likely to develop TB after being exposed?

“Boy, I don’t even know where you’d start with that,” Dr. Wilson says. Factors in play include intensity of exposure, duration of exposure, immune status, overall pulmonary status, smoking status, diabetes, and other risk factors. “It would be nice to have an algorithm to sort through all those issues. Perhaps there’s a role for artificial intelligence,” he says.

He likens it to trying to identify a patient’s risk of developing cancer. Having a gene mutation may be a risk factor, but that alone is not enough to make a sure prediction. “It’s not binary,” says Dr. Wilson.

Absent any immediate breakthroughs, Dr. Wilson makes a pitch for one more step laboratory professionals could take: perusing more journals, including those that might not appear on their regular reading lists.

Among his favorites, one is *Lancet Infectious Diseases*. Dr. Wilson cites an article from the January issue (Dorman SE, et al. 2018;18[1]:76–84), a large prospective study that looked at the Xpert MTB/RIF Ultra assay for detecting *M. tuberculosis* and rifampicin resistance. He notes, too, an article on laboratory testing for mycobacterial diseases published recently in *Clinical Microbiology Reviews* (Forbes BA, et al. 2018;31[2]:e00038-17). "Readers of CAP TODAY may not be thinking about these journals," he says, "but they have lots of good articles on TB testing." Though frequently aimed at specialists, they provide excellent context for what's happening in the field, he says. "It's a good way to know what might be coming next."
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