With syphilis rates rising sharply, syphilis tests a focus

Amy Carpenter Aquino

November 2017—Syphilis is making a comeback. Nearly 28,000 cases of primary and secondary syphilis, the most infectious stages of the disease, were reported in the U.S. in 2016—a 17.6 percent jump over 2015 and the highest reported rate since 1993. Cumbersome, subjective nontreponemal assays and the lack of a gold standard screening method lend complexity to the diagnostic process. But new nontreponemal assay options, including the first FDA-cleared fully automated treponemal/nontreponemal dual assay, may help stem the rising tide.

Dr. Fakile

"The best chances we have of catching people are usually in the primary and secondary phases when patients present with skin or mucosal lesions," says Yetunde F. Fakile, PhD, a microbiologist in the Centers for Disease Control and Prevention's Division of STD Prevention. If not identified and treated in the primary or secondary stage, syphilis enters a latent stage where there are no signs or symptoms, and the infection can go undetected for years, even decades, leading to other complications.

"The biggest issue we have is that there is no gold standard serologic test to diagnose syphilis," Dr. Fakile says. "There's no perfect test."

Laboratories in the U.S. perform one of two algorithms: a traditional algorithm, which is a nontreponemal assay (rapid plasma reagin or venereal disease research laboratory), followed by a treponemal assay (fluorescent treponemal antibody absorption test, *T. pallidum* particle agglutination, microhemagglutination assay, or enzyme immunoassay). The reverse algorithm flips the order and begins with a treponemal assay followed by a nontreponemal assay. Both assay types have pros and cons, and neither one can be used independently of the other.

"From a laboratory medicine perspective, one of the biggest challenges is that there is not a readily available, effective direct-detection method for *Treponema pallidum*," says John Schmitz, PhD, of the Department of Pathology and Laboratory Medicine, University of North Carolina School of Medicine, and director of the histocompatibility and clinical flow cytometry laboratories and associate director of the clinical microbiology/immunology laboratories, UNC Hospitals. "We can't culture it in the lab. There aren't antigen detection tests readily available, other than dark-field microscopy and molecular diagnostics, which aren't widely available. That presents a challenge when one is using an antibody response to diagnose an infection."

Of the two syphilis testing algorithms, Dr. Fakile says, "currently CDC does not recommend one algorithm over the other."

In April the CDC issued a multifaceted call to action on syphilis, one part of which urges researchers to "develop and bring to market novel syphilis tests to rapidly diagnose active infection." The CDC noted the sharp increase in cases of congenital syphilis, which rose 46 percent between 2012 and 2015, despite guidelines for screening of all pregnant women at the first prenatal visit. In its call to action, the CDC says: "The most commonly used tests require at least two sequential antibody tests in blood and do not confirm active syphilis infection. These blood tests are cumbersome, hard to interpret, unable to diagnose early infections, and may lead to treatment delays."

In development long before the call to action but released shortly after, the BioPlex 2200 Syphilis Total & RPR assay received Food and Drug Administration clearance in June. “It’s a novel and improved tool for detecting and managing syphilis,” says Chisanga Lwatula, PhD, global infectious disease product manager at Bio-Rad Laboratories.

It’s a dual assay, so treponemal and nontreponemal antibodies can be detected simultaneously. “It doesn’t matter what your algorithm is,” Dr. Lwatula says. “We’ve fully automated both assays and put them into a single test. You can choose in the software to report one or the other, or both at the same time.”

It removes the complexity that comes from interpreting the nontreponemal assay result and the extra step of reflexing to a second assay, because both assays are run simultaneously.

Dr. Lwatula

“The goal is to improve the workflow of the laboratory,” Dr. Lwatula says. “You’re also improving patient management because you’re getting those two results a lot faster.”

The automated RPR test protects against inaccurate readings of the nontreponemal card, Dr. Lwatula says. “The RPR is subjective; you have to interpret it by eye. Some of those card-based results are very difficult to interpret, especially the ones that are low-positive. One person might call it positive, and one person might call it negative. By automating the RPR test on the BioPlex 2200 system, you have objective results interpretation.”

The BioPlex 2200 Syphilis Total & RPR assay also provides full result traceability. With the process now automated, “your results are automatic, objective, and they’re directly sent to your laboratory information system.”

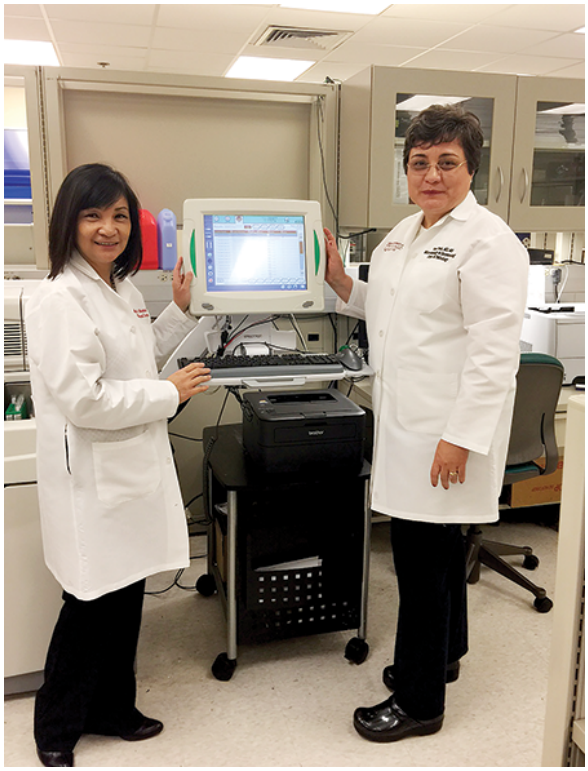
The CDC did not have data on the BioPlex 2200 Total & RPR assay when Dr. Fakile spoke with CAP TODAY in October. “As a laboratorian, I can tell you that we are interested in the performance of this assay,” she says.

At the University of Chicago Medicine, Vera Tesic, MD, and her colleagues are evaluating the BioPlex 2200 Syphilis Total & RPR assay for syphilis screening.

“The most exciting news for the laboratory professional is the availability of automated platforms for detection of both treponemal as well as nontreponemal antibodies,” says Dr. Tesic, assistant professor and assistant medical director of clinical microbiology and immunology labs, Department of Pathology.

The University of Chicago laboratories used to run the traditional algorithm, screening with the nontreponemal rapid plasma reagin followed by confirmation with fluorescent treponemal antibody absorption.

“We wanted to switch from the traditional algorithm to the reverse to reduce the manual labor for doing 30 to 40 RPRs every day,” Dr. Tesic says.



Dr. Vera Tesic (right) and immunology laboratory chief technologist Ana Precy Fajardo Abeleda at the University of Chicago, where they have reduced turnaround time for the syphilis diagnostic algorithm since implementing syphilis IgG testing on the BioPlex 2200 platform. They are now evaluating the BioPlex 2200 Syphilis Total & RPR assay.

Dr. Tesic and her colleagues implemented the reverse algorithm on the random-access BioPlex 2200 platform in early 2016. “We did syphilis IgG on BioPlex screening and then confirmed it with the RPR. The only time we would do the TP-PA [*T. pallidum* particle agglutination] would be when there was a positive syphilis IgG and the RPR was negative; then confirmation was needed to see if the IgG was false-positive or it was somebody who had syphilis in the past and was successfully treated. Hence the RPR in that case would be negative.”

She reports favorable results with the BioPlex 2200 platform and has high hopes for the newest assay, which she and colleagues are in the process of verifying.

“Since implementation of syphilis IgG testing on the BioPlex 2200 in our laboratory we significantly reduced turnaround time for the syphilis diagnostic algorithm, and we expect further reduction using the Syphilis Total and RPR assay,” Dr. Tesic says. The assay provides RPR titer up to 1:64.

The fully automated RPR capability of the BioPlex assay makes it only the second FDA-cleared assay with nontreponemal RPR automation available in the United States. Dr. Schmitz of UNC has been studying the AIX1000 fully automated RPR syphilis testing system, from Gold Standard Diagnostics, which the FDA cleared in November 2015.

“We looked at over 1,000 samples submitted to the laboratory and compared the automated system with our manual RPR method,” Dr. Schmitz says. He declined to comment on the performance of the AIX1000 assay pending publication of the UNC study.

He describes the AIX1000 automated system, which runs on the AIX1000 RPR automated processor, as “a pipetting device outfitted with optics, basically a digital camera.” It has software, he says, that can interpret the patterns in

the wells of the microplates that is used in this system to look for positive reactions in the RPR test system.

Another company, Arlington Scientific, hopes to have FDA clearance soon for its fully automated nontreponemal assay, which runs on the ASI Evolution syphilis analyzer. Mike LaDow, marketing director, cites a 2015 CDC study that found that the ASI RPR assay detected syphilis infection 14 days earlier than several treponemal assays.

LaDow

With automation coming to RPR testing, LaDow says, it will serve the high throughput needs of laboratories doing several hundred tests per night. “Because of the high costs of treponemal tests, they will serve their original purpose of verifying the nontreponemal result.”

He predicts a “tidal wave” of large companies working to develop automated nontreponemal assays to accommodate laboratories returning to the traditional algorithm.

“The nontreponemal system is an algorithm that has been used for 70 years, and a lot of people would agree it’s superior in terms of results for the clinician,” LaDow says. “If an RPR test for screening had been automated 12 to 15 years ago, treponemal tests would probably still only be used for confirmatory testing. The nontreponemal test is a better screening test.”

LaDow points to the fact that nontreponemal testing is required by the FDA for syphilis screening of all organ and tissue donations as further evidence that RPR testing produces better results.

Joanne Starkey, laboratory manager at VRL-Eurofins Laboratories in Denver, agrees that nontreponemal assays produce more accurate results. “You’re better able to distinguish between somebody who does not have a current, acute infection and somebody who has had the infection in the past. They are usually not going to be reactive using the nontreponemal whereas they could possibly come up with a reactive result on the treponemal test if they ever had the infection. A reactive result using a treponemal test doesn’t do you much good unless it’s paired with clinical information as far as does this person have any active symptoms or does their history indicate the possibility of an infection?”

Starkey

The problem with nontreponemal assays, though, is the risk of false-positives, she says. “There is a long list of parasitic infections or disease states or even autoimmune diseases that can cause a false-positive result with nontreponemal tests. They are relatively rare, but that is one of the hang-ups with the nontreponemal test; it is just throwing a net and catching what’s out there.”

Due to the nonspecific nature of nontreponemal tests, pregnant women and those with autoimmune disease and with some other infections that are not syphilis may have antibodies that can be picked up by a nontreponemal assay, Dr. Fakile of the CDC says. “If you are reactive to a nontreponemal assay, it does not mean you have

syphilis. It could be a nonspecific reaction. And if you use a trep-only test and it comes up reactive, it could be past infection, or it could be recent infection. So you have to investigate further."

Starkey's reference laboratory is running more than 200 syphilis tests per day, mostly from cadaveric samples for organ and tissue donation screening, on the FDA-approved ASiManager assay. Reactive results for syphilis infection are confirmed with the Trinity Biotech Captia Syphilis (*T. pallidum*)-G.

"Moving away from the subjective assays and more toward the objective assays like Captia is something I think the industry would like to see," she says.

As far as which syphilis testing algorithm is preferred, "I don't think there's an easy answer to that, unfortunately," Dr. Schmitz of UNC says.

"We've done some comparison studies here on reverse versus traditional algorithms, and we certainly do pick up more positives using a reverse algorithm approach," he says. "I can't draw a firm conclusion yet about performance, but we certainly pick up more positives that are confirmed by a second, different treponemal assay."

The sensitivity of the treponemal assay, performed first, gives it the ability to detect latent infection that might be missed using the traditional screening algorithm. "It's been pretty clearly demonstrated that the treponemal screening test may be slightly more sensitive in very early infection, and certainly more sensitive in latent syphilis, where the sensitivity of the nontreponemal test can decline over time," Dr. Schmitz says.

The lack of specificity in the treponemal test can be a drawback, though. "It presents the clinician with the scenario of having to determine if that confirmed reactive treponemal test and nonreactive nontreponemal test is due to past treated or untreated syphilis." If a laboratory has a positive treponemal assay result followed by nonreactive nontreponemal assay result, a second, different treponemal assay must be performed.

Dr. Schmitz

Nontreponemal assays are also preferred for monitoring therapeutic efficacy, Dr. Schmitz says. "We don't want to use a treponemal screen or reverse algorithm in those cases. We want to go to a nontreponemal test and do titers to look for declines in titer with effective treatment."
In higher prevalence populations, the nontreponemal test is used to assess whether a patient has been re-infected by looking for an increase in titer, Dr. Schmitz says. "That isn't going to be typically detected with a treponemal antibody screen, because those are qualitative tests."

test, Dr. Fakile says, "where if I test an individual sample, I can tell you, 'This is it, the person has active syphilis.'"

"We are very focused on trying to find new tests that can be developed to get to the bottom of this diagnostic challenge, and then find tests that are much more rapid, closer to the patient, and do have good sensitivity and specificity."

In the U.S., there is only one rapid point-of-care test, Dr. Fakile says, the Syphilis Health Check (SHC) by Trinity Biotech. "The CDC is still looking at rapid point-of-care testing with a lot of interest and trying to gather as much data as we can, trying to reach out to people or programs that may be using this test," Dr. Fakile says. Another rapid test is the DPP Syphilis Screen and Confirm assay, from Chembio Diagnostic Systems, which is a dual nontreponemal and treponemal POC test. It does not have FDA clearance.

“We are considering a lot of other new assays which are just entering the U.S. market,” Dr. Fakile says, “and trying to understand the performance characteristics so we can properly identify the patients early, treat them and their contacts, and stem the rise of syphilis.”
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