New tests, new wrinkles in HIV algorithm

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September 2017—Three years—including a total eclipse of the sun—have sped by since the Centers for Disease Control and Prevention and the Association of Public Health Laboratories recommended a new HIV diagnostic testing algorithm for laboratories. In 2014, the algorithm was seen as bringing HIV test ordering up to speed with the advances in HIV test technology and increasing the accuracy and reliability of HIV screening and diagnosis. Have laboratories made the adjustment, and is the CDC/APHL algorithm proving workable and worthwhile?

Dr. Philip Peters of the CDC Division of HIV/AIDS Prevention. He and others spoke recently about the 2014 HIV testing algorithm and new challenges.

Philip Peters, MD, believes the answers are yes—although the answers aren't simple. "Having a robust HIV testing program is really a vital part of HIV prevention," says Dr. Peters, medical officer of the CDC Division of HIV/AIDS Prevention, in an interview with CAP TODAY. Laboratories are successfully adopting the algorithm, but implementation has been met with challenges. "Sometimes newly approved tests don't neatly fit into the existing framework or algorithm for HIV testing," he says.

The CDC/APHL algorithm has won widespread adoption and helped unseat the 1980s-era Western blot as the confirmatory assay of choice, probably for good. But in practice, features and limitations of some of the commercially available tests have made the algorithm somewhat tricky to follow, and as new tests have emerged, they have complicated decision points in the algorithm.

Speaking in a panel on how laboratories are dealing with the CDC algorithm, at the AACC annual meeting in August, Dr. Peters and co-presenters outlined the state of current HIV testing relative to the algorithm and discussed some of the potential complications that new HIV diagnostic tests can create, as well as prospects for continued progress in HIV testing. If the AACC audience is any indicator—periodic online surveys during the presentations allowed laboratorians there to vote on user devices—adoption of the algorithm is proceeding but challenges remain.

In brief, the CDC/APHL laboratory algorithm calls for an initial test by an antigen-antibody combination assay. The antigen-antibody combination assays are the Abbott Architect HIV Ag/Ab Combo, Bio-Rad GS HIV Combo Ag/Ab EIA, Siemens Advia Centaur HIV Ag/Ab Combo, and Bio-Rad BioPlex 2200 HIV Ag-Ab; the Roche Elecsys HIV combi PT Immunoassay (approved by the FDA on June 21) is the most recent addition to the list.

If the antigen-antibody combination test is positive, the next step is an antibody-based differentiation assay that differentiates between HIV-1 and HIV-2. Bio-Rad's Geenius HIV 1/2 Supplemental Assay or, until recently, Bio-Rad's Multispot HIV-1/HIV-2 Rapid test have been commonly used. If there's a discrepancy between the initial screening antigen-antibody test and the supplemental assay, the next step is a nucleic acid test, and the only one currently FDA approved for diagnosis is the Hologic Aptima HIV-1 RNA Qualitative Assay. Positive samples at that stage are considered acute HIV-1 infection.

FDA approvals of diagnostic tests and laboratories' installed instrumentation have both influenced recent trends in HIV testing. In the first years after the 2014 HIV testing algorithm recommendation was issued, it was something of a challenge to get laboratories to use the antigen-antibody combination assays, says Teal Clocksin, MS, technical specialist for TriCore Reference Laboratories, Albuquerque, NM.

After the BioPlex and Advia assays were approved by the FDA in 2015 to detect HIV-1 antibodies, HIV-2 antibodies, and HIV-1 p24 antigen, Clocksin says, "a big portion of what was before a difficulty was essentially eliminated. Many labs already have the Siemens Centaur, which is one of the instruments offering HIV combination testing. And that really opened the door for lots of additional labs to start combination testing."

"When you have more tests on the menu than just HIV and you're doing lots of vitamin D or thyroid testing on your instrument, it would be a big decision to switch up everything in your lab just based on one test," notes Dr. Peters, who has led several studies on acute HIV infection and HIV transmission. "But now that most of the manufacturers are offering the combo test, it's becoming a lot easier." About 90 percent of the AACC panel's audience who were laboratorians reported using a lab-based antigen-antibody combination immunoassay.

Only about 65 percent of the panel's audience reported using the second assay the algorithm calls for, a differentiation assay, though many audience members were not from the United States. Dr. Peters does not find this response surprising. "It hasn't been approved as long, and unlike the multiple antigen-antibody tests available from different manufacturers, there's only one manufacturer [Bio-Rad] that makes the differentiation assays."

The third step of the algorithm shifts from immunoassay to nucleic acid testing. Several instrumented NAT assays are approved for monitoring patients' viral loads, but only the Aptima, not automated, is FDA approved for diagnosis. In asking for audience votes at AACC, "We were interested in knowing, first, if people had access to viral load assays within their lab, and, second, if they were using it only for monitoring or using it for help with diagnoses in the case where they have internally validated the test," Dr. Peters says.

"But there is a tension between the NAT assays because the Aptima is FDA approved for HIV diagnosis but not widely available in clinical labs, so it would be a send-out test, whereas the viral load assays are present in a lot of clinical labs," he notes. Among the audience members in the AACC session, about half said they were using their nucleic acid test for both diagnosis and monitoring. "That's about what I expected, because the lab can get a quicker turnaround time than if they have to send it out. But anecdotally, I heard people in the audience saying it would be great if these viral load assays were also FDA approved for HIV diagnosis so they wouldn't have to validate these tests for an off-label use."

The most difficult piece of the algorithm at this point is the nucleic acid test, Clocksin says. "The event where you have a positive combo result and a negative confirmation result happens so rarely that it doesn't make sense for a lot of labs to bring in the Aptima just for that purpose." As a high-complexity test under CLIA, "Aptima requires specialized training, and most hospitals or even large institutions don't have that capacity," so it tends to be a reference test.

Although other nucleic acid tests exist—including the Roche Cobas AmpliPrep/Cobas TaqMan HIV-1 and Abbott

RealTime HIV-1—they are likely to continue to be used only for viral load testing to monitor treatment. “They may be just as sensitive for detecting an acute infection, but manufacturers don’t have a large incentive to get an additional diagnostic intended use designation from the FDA because it’s such a rare event,” Clocksin says. Single-use nucleic acid tests available only outside the U.S. include the Cepheid GeneXpert, Alere q point-of-care analyzer, and SAMBA (simple amplification-based assay) system. The Roche Cobas Liat Analyzer is a single-use nucleic acid test now in the pipeline for FDA approval.

Clocksin

Longer turnaround time is the downside of sending out samples for NAT testing. But other factors in turnaround have improved since the 2014 updated algorithm, Clocksin points out. That’s largely because the algorithm recommended against using Western blot. Automation, employing track systems, has also shortened the testing process.

A key priority remaining now, Clocksin believes, is to get all laboratories on an antibody-antigen combination screening assay and an in-house confirmation assay so they can shorten turnaround time as much as possible. “Then the largest challenge is to try to expand NAT so it’s more readily accessible to more labs, and that comes down to additional FDA approvals and a desire by the manufacturers to pursue those.”

The advanced features of some new HIV tests could have an impact on the laboratory algorithm, said AACC co-panelist Laura Wesolowski, PhD, an infectious disease epidemiologist with the CDC and coauthor of the CDC/APHL Laboratory Testing Guidance.

She pointed to the BioPlex 2200 HIV antigen-antibody assay as an example. “It’s a multiplex immunoassay that is a random-access test. It can be used to detect other pathogens and can be used with plasma or serum. It differentiates between p24 antigen, HIV-1 antibody, and HIV-2 antibody and gives results in about an hour.” This contrasts with a single reactive result coming from the antigen-antibody test. But “should we go directly to the RNA step after a p24-only antigen is positive?” That question hasn’t been answered yet, she said. “That’s one reason we’re asking for additional published data specific to this test.”

The Alere Determine HIV-1/2 Ag/Ab Combo test presents complications as well. “It’s CLIA moderately complex when it’s used with serum or plasma. And it distinguishes HIV-1 p24 antigen from antibody and doesn’t differentiate between HIV-1 antibody and HIV-2 antibody. It’s interpreted subjectively and it doesn’t have a reader.” This test was not recommended in 2014 as a screening test in the algorithm because little data was available at that time.

Supplemental tests, too, may have an impact on the algorithm, Dr. Wesolowski said. “One test that adds complexity is Bio-Rad’s Geenius, which replaced the Multispot, which is no longer manufactured. Geenius is the only approved supplemental antibody assay that differentiates HIV-1 from HIV-2. It runs in less than 30 minutes in repeatedly reactive specimens and uses an automated reader.”

Geenius produces eight final results based on two analytes, the HIV-1 and HIV-2 antibody. Three of these results were not generated by Multispot. The first was HIV-2 positive with HIV-1 cross-reactivity. “Antibody to the HIV-2 is confirmed and only one HIV-1 envelope protein is present, which precludes confirmation of HIV-1,” Dr. Wesolowski said. “These should be considered HIV-2 positive and the person should be sent for clinical follow-up.”

The second new result Geenius produced was the HIV-2 indeterminate. “In such a case, where there is reactivity to one of the two HIV-2 antigens but not both gp36 or gp140, the manufacturer recommends you repeat the results. But a lot of times these results will not repeat. And in that case, we would have an HIV-negative Geenius and move on to the HIV-1 NAT step in the algorithm. But since we have observed some HIV-2 indeterminate results in persons with early HIV-1 infection, an HIV-2 indeterminate that is repeatedly detected should also proceed to HIV-1 NAT. If that test is negative, you should either reflex to testing with a validated HIV-2 test or repeat testing from the top of the algorithm in two to four weeks.”
The third result obtained by Geenius but not by Multispot was the HIV indeterminate. “In this case, neither HIV-1 nor HIV-2 can be ruled in or out. And the sequence of tests is again to begin with an HIV-1 NAT, and if it’s negative, conduct additional testing with a validated HIV-2 test or repeat testing within two to four weeks.”

The way that Geenius tests are reported can flummox clinicians, since both the final determination and results for HIV-1 and HIV-2 are included. “The 1 and 2 are included in brackets on their results sheet, and that can make reporting complicated.” For example, if the final determination shows an HIV-1 positive, inside the brackets it may indicate ‘HIV-2 indeterminate,’” Dr. Wesolowski said. “Although the Geenius reader is programmed to ignore the HIV-2 reactivity and report it as HIV-1 positive, if all results are reported it can be confusing to a clinician to see the HIV-2 indeterminate and then be told to ignore that result.”

To address these issues, the APHL produced a suggested reporting language document that reflects all possibilities for outcomes with the Geenius test (available at www.bit.ly/APHL-reportinglanguage). From the CDC’s standpoint, she said, “We are interested in seeing publications related to BioPlex, Geenius, and Determine performance with serum or plasma, as well as the performance of simplified NAT tests, especially in the context of the lab algorithm.”

Small laboratories can have special challenges in implementing the algorithm, Dr. Wesolowski said. “At any step in the algorithm, some laboratories are sending out the test, which may produce delays and potentially could delay treatment in infected persons. And some small-volume labs may want to do a rapid, single-use antigen-antibody test, although that has been shown to be less sensitive for early infection.”

In addition, “there are not very many options for the second and third steps in the algorithm,” since there is only one FDA-approved HIV 1/2 differentiation test. “And we have heard that it can be costlier than its predecessor, and it can produce HIV-2 indeterminate results not indicative of infection.” If a laboratory chooses to validate another supplemental antibody test, it should ensure orthogonality with the screening test, Dr. Wesolowski said.

She noted two other concerns the CDC has heard. Providers are sometimes confused as to whether they are ordering antigen-antibody tests or an IgG/IgM test. A separate issue is that sometimes a new specimen is indicated if the initial one used for screening doesn’t meet the NAT requirements by the time it gets to that step in the algorithm, leading to a delay in obtaining results. Interestingly, in an on-the-spot survey of the AACC audience, laboratorians reported experiencing about the same levels of challenges with the algorithm for the combination test, the supplemental test, and the nucleic acid test, with 25 percent reporting no significant challenges.

The CDC has tested the laboratory algorithm in various scenarios, said AACC co-panelist Silvina Masciotra, MSc, who works on the CDC HIV Diagnostics and Molecular Epidemiology Team in the Laboratory Branch. She outlined a theoretical example arising from an HIV-1 outbreak in a high-risk population in a large city. Investigators collected whole blood samples from people who inject drugs and send the tubes to a central laboratory for HIV testing following the CDC/APHL algorithm.

“In the central laboratory, the BioPlex 2200 antigen-antibody test is performed, followed by Geenius, if they’re repeatedly reactive, and reflexed to NAT when results between screening and supplemental antibody tests are discordant,” said Masciotra, a research microbiologist who has published several studies evaluating HIV testing technology. In the CDC study, about 30 percent of the samples showed reactivity only in p24, but the results from the Geenius showed no reactivity. “We were detecting p24, one of the first viral markers to appear and detected

during acute infection where antibodies are not yet present," Masciotra said. "Geenius is an IgG-sensitive assay, so absence of IgG reactivity is somehow expected. These are preliminary results to determine whether a specimen that is p24-only reactive can go straight to NAT in the future."

A second scenario she described also involves the outbreak in the large city. Here, "there is a shortage of BioPlex 2200 reagents but there are some Determine Combo tests available to test plasma." The audience was asked whether the Determine Combo and BioPlex would produce the same number of reactive responses or a different number, and most responded that BioPlex will have more reactive results than Determine Combo. "We tested the BioPlex and Determine Combo in parallel, and BioPlex indeed detected more reactive results than the Determine Combo. So the use of the Determine Combo might help in certain circumstances, but it will not be detecting as many samples in that early stage of HIV infection," Masciotra concluded.

Laboratories may want to determine whether they are ready to support HIV testing for patients on PrEP, the pre-exposure prophylaxis HIV prevention option for at-risk persons who are still negative, Dr. Peters said in his AACC talk. PrEP is a method to prevent HIV infection whereby persons take a daily oral pill that contains tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC). Several studies have shown that the risk of acquiring HIV is as much as 92 percent lower in persons who are taking PrEP consistently.
HIV testing is an important part of providing PrEP. Testing should include an evaluation for acute HIV infections, and testing needs to be repeated at least every three months while persons are taking PrEP, Dr. Peters said.

In some situations, a person will become HIV-infected despite taking PrEP, and that raises interesting considerations of which laboratories should be aware. In one example he cites, a person had infection at such an early stage that only the HIV RNA test was reactive, and it was at a very low level. But the clinician had access only to a rapid antibody test that was not reactive, so PrEP was initiated. "Unfortunately, drug resistance developed because the PrEP medications cause some viral suppression, but not enough—so the virus is able to generate mutations and resistance against the PrEP medications."

Basically, if laboratories are ready to perform HIV testing in general, then from a technical standpoint they are ready to provide HIV testing to support PrEP, in Dr. Peters' view. "But it's really a matter of talking to clinicians who are providing PrEP about the type of assays they feel are important to provide to their patients to ensure these assays are available. Then also consider the impact that PrEP would have on an early HIV infection because of its potential to cause viral suppression."

In his AACC talk, Dr. Peters gave other hypothetical examples of "current HIV testing dilemmas" that can crop up with current HIV test technology. In the first, the "puzzled physician," a patient with no risk factors had a false-positive screening; the antigen-antibody screening combination test result was positive, the differentiation assay was negative, and the HIV-1 RNA NAT was also negative.

"This is something that does happen, because although these tests are highly specific, approaching 99.93 percent specificity, we do so much HIV testing that we still have about one out of every 1,000 or 3,000 test results come back as false-positives." This can excite and confuse clinicians, Dr. Peters warns, and laboratories need to be prepared to explain.

The "curious clinic" posed a different dilemma. Here the medical clinic contacts the lab regarding HIV testing for their new PrEP clinic and reports it has received free oral fluid HIV tests from the health department. "Oral fluid HIV testing is not recommended with PrEP," Dr. Peters says, because if somebody becomes HIV-infected while taking the PrEP medication, they can have a delayed seroconversion that is most markedly seen with oral fluid tests.

"So laboratories should advise clinicians doing HIV testing for patients on PrEP to use either a fingerstick or blood specimen."

A third case involved a PrEP clinic that called the lab for clarification of results. "They have a negative rapid test. They're reactive on their combination antigen-antibody and they're HIV-1 reactive on their differentiation assay,

the Geenius test. The patient, who has been receiving PrEP, has less than 400 copies per mL on the HIV viral load, has no symptoms, and reports condomless anal sex with multiple new partners." These results are consistent with an early HIV infection complicated by PrEP medication that is suppressing the viral load, something that can occur if people are taking their medication consistently, Dr. Peters says. "It's not always below the level of quantification, but there is going to be suppression of the viral load."

In another hypothetical situation, an inpatient hospital service calls the lab for clarification of a combination antigen-antibody result that is repeatedly reactive and a type differentiation assay result, Geenius, that is HIV indeterminate. "The patient was admitted with pneumocystis pneumonia with a CD4 cell count of 145, so they have AIDS, and the HIV-1 RNA test detects HIV-1 with quantification pending." In the United States, where HIV-2 is rare, if the HIV-1 RNA test is reactive, then the person would be diagnosed with HIV-1. The differentiation test was not able to differentiate the antibody result and gave an indeterminate result, Dr. Peters says, but "without any further history of HIV-2 risk factors, you would not be obligated to rule out HIV-2 infection in this situation."

Notable progress has been made recently in the fight to control HIV infections, Dr. Peters points out. Between 2011 and 2014, new HIV infections in the U.S. did something significant: They declined, by 18 percent. This news indicates that HIV prevention efforts are working, he says. "There's still a lot of work to be done, but I think it's very encouraging that we're starting to see declines in the number of new infections."

The role of laboratories in these declines is significant, Dr. Peters says. "It's hard to tease out what to attribute to the different parts of HIV prevention—what proportion is due to diagnoses or to people's use of condoms, etc. But HIV testing is an important component of our relatively new approach to HIV prevention. If you test HIV positive, taking antiretrovirals can have up to a 96 percent reduction in HIV transmission. If you test HIV negative, taking PrEP can have up to a 92 percent reduction in the risk of acquiring HIV."

"We see HIV testing as the linchpin that really brings all of that together. Before you understand what type of prevention you can provide to someone, you need to know their HIV status. If they are positive, we know making that diagnosis early in the infection, before further transmissions can occur, is not all the work that needs to be done. But it's a critical first step before somebody can get into treatment."

Having a robust HIV testing system is a vital part of HIV prevention, he says. "That's something we try to emphasize for laboratorians—that they are a crucial partner in HIV prevention, they are one of the reasons why we are seeing this decline, and we are looking forward to continuing to expand access to HIV testing."

The CDC is continuing to tackle HIV testing from several angles, Dr. Peters notes. The DETECT study, a research contract with the University of Washington and Seattle/King County public health agencies, is looking at how well different tests detect infection during the earliest phases of acute infection. In a recently published preliminary report, Project DETECT researchers reported identification of one individual who was serially false-positive on only one test and identification of three persons in the early stage of HIV infection. The study is currently enrolling participants. The researchers plan to identify more newly infected individuals at the point of care and follow them to compare test performance when using unprocessed fingerstick blood and oral fluid. They also plan to add point-of-care nucleic acid testing in the next year, and expand recruitment of people with known HIV infection to stabilize sample size for estimates of test sensitivity.

The agency is also cosponsoring a conference on HIV diagnostic testing that will take place in 2019. "It's a little ways off. But we want people to start thinking about that conference and what they want to discuss and present at it," Dr. Peters says. Finally, the CDC is offering a new funding opportunity. Beginning in 2018, a large grant will be distributed to state and local health departments that will provide funding for HIV surveillance and HIV prevention. "And a big part of those activities will be supporting HIV testing and supporting implementation of PrEP," he says.
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