Understanding and Utilizing New Techniques For HIV Testing

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Approximately 40,000 new HIV infections occur every year in the United States. Of even greater concern, however, is the U.S. Centers for Disease Control (CDC) estimation that between 180,000 and 280,000 people in the United States are infected with the virus and don't know it. In turn, there is a sizeable population of HIV-infected individuals who are unknowingly putting their own health at risk and likely contributing to the spread of HIV. Another concern is the fact that between 27,000 and 30,000 of blood tests conducted at publicly funded testing sites are positive for HIV antibodies. However, approximately 31% of those who test positive do not return to the testing site to receive their results.

Because of these and other startling statistics, the CDC has launched a new HIV testing, counseling, and referral initiative—dubbed *HIV Prevention: New Strategies for a Changing Epidemic*—that aims to reduce barriers to early diagnosis of HIV and increase access to quality medical care, treatment, and ongoing prevention services. One of the strategies outlined in this initiative is to make HIV testing a routine part of medical care, by helping to familiarize clinicians with the development and availability of new diagnostic technologies, including rapid assays. Rapid assays have been developed to make point-of-care (POC) HIV testing feasible and to greatly reduce the number of persons who do not learn their HIV status by providing immediate results.

The CDC also recognizes that, if they and other prevention groups are to be successful in stemming the tide of the epidemic, it is necessary to understand the ever-changing trends in HIV transmission. This requires monitoring the incidence of new HIV infections in various at-risk populations. To do this, the CDC has been implementing programs to take advantage of new testing strategies, including sensitive/less-sensitive antibody testing algorithms to identify newly infected individuals from among the scores of persons testing positive for HIV.

Commercially Available Rapid Assays

More than 60 rapid HIV tests have been developed and used in various countries, most notably in developing nations. In the United States, the U.S. Food and Drug Administration (FDA) have approved only four assays. The first assay, Recombigen HIV-1 LA, was a latex agglutination test. Unfortunately, its results were often difficult to interpret—even among the most seasoned laboratory technicians—and the test was subsequently withdrawn from the U.S. market because of poor performance. In 1992, the FDA approved the Single Use Diagnostic System for HIV-1 (SUDS), which is not well suited for POC use, given that its reagents require refrigeration and multiple steps in conducting the test are required. Two rapid assays have recently won FDA approval: OraQuick Rapid HIV-1 Antibody Test (Orasure Technologies, Inc.) and Reveal Rapid HIV-1 Antibody Test (MedMira, Inc.). According to the CDC, OraQuick is the first rapid assay to become available in the U.S. that is truly suitable for POC testing.

OraQuick Rapid HIV-1 Antibody Test

The OraQuick test is approved for use with fingerstick and anticoagulated whole blood specimens. "However," Dr. Bernard Branson pointed out, "it is also designed to be used with serum or oral fluid specimens." It is intended for use as a POC test, in medical and nonmedical settings. It can also be performed in a separate laboratory after the specimen has been obtained.

To conduct the test, a vial of developer solution is placed in a plastic stand (see Figure 1). The reusable stand holds the test device at the correct angle to ensure accurate test results. A drop of blood is collected with a small plastic loop from the punctured finger or blood tube and stirred into the vial of developer solution. The OraQuick device is inserted into the developer vial where it remains until the results are read. Test results must be read no sooner than 20 minutes but no later than 40 minutes after the device is added to the developer solution.

The test result is read directly from the OraQuick device (see Figure 2). If only one reddish band appears at the control (C) location the test result is negative for HIV-1 antibodies. If two reddish bands appear, one at the control (C) location and one at the test (T) location, the test is "reactive"—that is, a preliminary positive for HIV-1 antibodies. However, if no band appears at the C location, if any bands appear outside the C or T locations, or if a pink-red background appears in the device window, the test is invalid and must be repeated.

Because the OraQuick test includes an internal positive control, it is not necessary to run external control specimens with each test. However, positive and negative external controls should be run by each new operator prior to performing testing on patient specimens, whenever a new lot of test kits is used, if the conditions of testing or storage (e.g., temperature) fall outside the range recommended by the manufacturer, and at periodic intervals specified in the laboratory's quality assurance program.

The OraQuick assay boasts a sensitivity of 99.6% and a specificity of 100%.

More information on the OraQuick Rapid HIV-1 test can be obtained through OraSure's website (http://www.orasure.com).

Reveal Rapid HIV-1 Antibody Test

The Reveal test, another rapid test for HIV antibodies, is also intended for use as a POC test, but because it can be used with only serum or plasma, it requires some laboratory equipment. The Reveal test does not contain an internal procedural control. External controls—known HIV-positive and HIV-negative specimens—must be run with each test or batch of tests to monitor test performance. A detection agent and positive and negative controls, which are supplied with the test, must be reconstituted with buffer solution. These reagents, each sufficient for five tests, can be stored via refrigeration for up to seven days after they are reconstituted.

To do the test, a blood sample is drawn from a vein and centrifuged to separate the red blood cells from the serum or plasma. A buffer

solution is placed in the test cartridge and allowed to absorb. The serum or plasma specimen is added to the test cartridge and allowed to absorb, followed by the addition of a buffer. The detection agent is then added to the test cartridge, again followed by the addition of a buffer.

"The Reveal assay might be more useful to those who have onsite laboratories," Dr. Branson pointed out. "Laboratory personnel can handle specimens much in the way they handle other specimens collected by venipuncture."

The test result is read directly from the cartridge as soon as all the solution is absorbed. A red dot on the test cartridge indicates the test result is "reactive," that is, preliminary positive for HIV-1 antibodies. No red dot indicates that the test is negative for HIV-1 antibodies. A pinkish-red background throughout the window of the cartridge indicates the test is invalid and must be repeated.

The Reveal Rapid assay has a sensitivity of 99.8% and a specificity of 99.1% if serum samples are used, or a specificity of 98.6% if plasma samples are used. "This specificity is lower than the OraQuick assay, so the issue of false-positive results are of concern."

More information about the Reveal Rapid HIV-1 Antibody Test can be accessed through MedMira's website (http://www.medmira.com).

Requirements for Performing Rapid HIV Tests

ANY MEDICAL OFFICE, CLINIC, OR ORGANIZATION THAT PERforms a rapid HIV test to provide results to patients is considered to be a laboratory under the Clinical Laboratory Improvement Amendments of 1988 (CLIA). As a result, all laboratories must comply with the regulations of the CLIA Program and with any applicable state requirements.

Sale of rapid HIV tests is restricted to clinical laboratories that have an adequate quality assurance program where persons who use the test will receive and use the instructional materials provided with the tests. The FDA also requires that persons tested with the OraQuick and Reveal tests receive the "Subject Information" pamphlet provided with the test. Details about other restrictions that apply to the rapid HIV tests are outlined in the package inserts provided with the test kits.

Currently available rapid HIV tests are either "waived" or categorized as "moderate complexity" under the CLIA program. CLIA requirements for laboratories differ depending on the category and complexity of the test.

The OraQuick Rapid HIV-1 Antibody Test is a CLIA-waived test. For waived tests, there are no federal requirements for personnel, quality assessment, or proficiency testing, although the tests must comply with state and local regulations and laws. In turn, waived tests can easily be done in traditional laboratories or clinical settings, and also in settings such as doctors' offices, HIV counseling and testing sites, mobile vans, and health fairs.

To perform only waived tests, an organization must obtain a certificate of waiver from the CLIA program (or be included with a CLIA-certified laboratory under a multiple site exception, if authorized by CMS) and follow the manufacturer's instructions for the test procedure. [EDITOR'S NOTE:

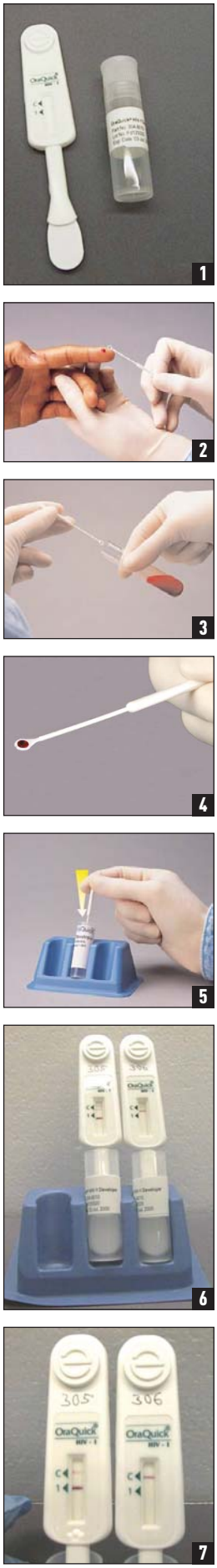

Figure 1. **Performing an OraQuick Rapid HIV-1 Antibody Test**

A sample OraQuick Rapid HIV-1 Antibody Test is shown here (image 1). To collect a specimen for the test, either touch the collection loop to a fingerstick blood droplet (image 2) or use standard phlebotomy collection procedures for whole blood with the following test tubes: EDTA, Sodium Heparin, Sodium Citrate, or ACD Solution and dip the collection loop into the test tube (image 3). Five microliters of whole blood should adhere to and fill a single collection loop (image 4). Insert the loop and stir the specimen in the vial of developer solution (image 5). The OraQuick device is inserted into the developer vial where it remains until the results are read (image 6). Test results must be read no sooner than 20 minutes but no later than 40 minutes after the device is added to the developer solution (image 7).

Source: Orasure, Inc.; U.S. Centers for Disease Control and Prevention

In New York State, this waiver must be obtained from the New York State Department of Health Wadsworth Center as a Limited Service Laboratory. For information on requirements and application procedures, facilities may contact CLEP at (518) 485-5378 or visit the website at: http://www.wadsworth.org/labcert/clep/clep.html *and click on the "Permit Application Materials" link.*]

The Reveal Rapid HIV-1 Antibody Test is categorized as a moderate complexity test. A laboratory that performs moderate complexity tests must register with the CLIA program and meet specific CLIA quality standards for personnel, quality assessment, proficiency testing, and inspections.

Interpreting and Confirming Rapid HIV Test Results

THE RESULTS OF RAPID HIV TESTS ARE INTERPRETED THE same way as the results of other HIV screening tests. A non-reactive result from a single test is considered negative. However, persons whose test result are negative may have been exposed to HIV within the past three months and may not yet have developed detectable antibodies to HIV. These individuals may have a negative test result. A repeat test after three months is recommended for persons with a negative rapid HIV test.

A single reactive result from a rapid HIV test is considered to be a preliminary positive result. The person receiving the test result can be told that the result is a "preliminary positive." All preliminary positive rapid tests must be followed up with another type of test, either a Western blot or immunofluorescence assay, to confirm the result. The person is considered HIV-positive only if the confirmatory test result is positive. A small percentage of specimens give indeterminate results in the confirmatory test. If this happens, the test should be repeated after one month.

"In many outreach settings, testing staff are obtaining oral fluid specimens for Western blot testing," Dr. Branson explained. Because the rapid test may be more sensitive than the traditional EIA screening test, an HIV-infected

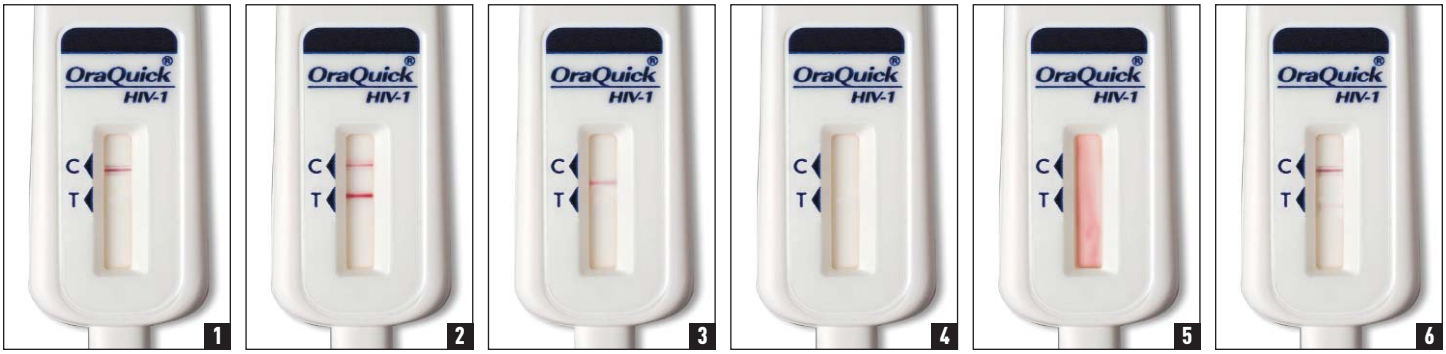

Figure 2. Reading and Interpreting an OraQuick Rapid HIV-1 Antibody Test

Six possible results using the OraQuick Rapid HIV-1 Antibody Test. A nonreactive test will yield a line in the control (C) area and no line present in the test (T) area (Image 1). A nonreactive test result should be interpreted as negative for HIV-1 antibodies. A reactive test will yield a line in the control area and a line in the test area (Image 2). A reactive test result should be interpreted as a preliminary positive for HIV-1 antibodies and must be confirmed. Also shown here are three examples of invalid test responses (Images 3–5), which require that a second test be performed. A weakly reactive test will yield a line in the control area and a weak line present in the test area (Image 6). Follow-up testing is recommended to confirm an initial weakly active result.

Source: Orasure, Inc.; U.S. Centers for Disease Control and Prevention

person may have a positive rapid test and a negative EIA test. "Therefore," Dr. Branson added, "it is important that when you send a specimen for follow-up testing, you insist that they do a confirmatory HIV test, even if the EIA is negative. In fact, it is unnecessary for laboratories to perform an EIA test after a person has had a preliminary rapid test. Most laboratories are used to the usual algorithm—negative EIA results are reported as such and Western blot tests are not performed. However, there have been several instances reported anecdotally in which the OraQuick test was positive, the laboratory EIA was negative, and the confirmatory Western blot test was positive. So when you submit a test for confirmatory Western blot testing, it is important to state that the person has already received a positive screening HIV antibody test and requires a confirmatory test irrespective of the EIA test result."

New Opportunities for Confusion

HOW IS IT POSSIBLE FOR A RAPID ASSAY TO YIELD A POSITIVE AND A LABORATORY-CONDUCTED EIA to yield a negative result? The answer can be found in the fact that there are six FDA-approved EIAs, which vary considerably in their ability to detect recent HIV infections.

The six approved assays are: the Abbott HIVAB HIV-1/HIV-2 (rDNA) assay, the Genetic Systems HIV-1/HIV-2 Peptide assay, the Genetic Systems HIV-1/HIV-2 Plus assay (approved this past summer), the Genetic Systems rLAV/HIV-1 assay, the Vironostika HIV-1 Microelisa assay, and the Vironostika HIV-1 Plus O Microelisa assay (also approved this past summer).

Both OraQuick and the Abbott EIA detect IgM antibodies and may be positive as soon as 14 days after acquiring infection. The Genetic Systems HIV-1/HIV Peptide and the Vironostika assays are less sensitive and capable of detecting antibodies to HIV approximately 21 days after exposure. The Genetic System rLAV/HIV-1 assay becomes reliable 23 days after exposure. Confusing matters further, the two FDA-approved Western blot assays—marketed by BioRad and Cambridge Biotech—may yield negative or inconclusive results when an OraQuick assay or Abbott EIA has yielded positive results.

In terms of dealing with the confusion surrounding rapid assay and confirmatory testing, Dr. Branson stressed that clinicians need to be familiar with the various tests being used, as they are different. "Tests should be chosen carefully and confirmatory testing should include both EIA and Western blot," he urged.

Testing and the Epidemiology of Recent HIV Infections

THE CDC'S STATED GOAL OF REDUCING NEW INFECTIONS IN THE U.S. BY 50% in five years will require a comprehensive approach, a key element of which will be identifying populations with high incidence of recent HIV infection. "If we are to have a major impact on the spread of HIV, we need to focus on new infections," Dr. Branson said. "Monitoring the incidence of HIV will also help us to direct HIV subtypes or patterns of drug resistance in newly infected individuals. Monitoring the incidence of HIV infection will also be important in terms of identifying cohorts for vaccine trials."

There are a number of laboratory-based methods that can be used to identify individuals who have recently been infected with the virus. HIV-RNA and p24-antigen testing for the purpose of detecting acute HIV infection have been discussed extensively in the pages of *The PRN Notebook* and have become popular among clinicians, particularly those caring for at-risk patients. HIV-RNA testing in particular is being used in parts of the United States, notably North Carolina, to monitor HIV incidence rates (see: "Feasible Primary HIV Infection Screening: The North Carolina Experience," an article based on a lecture by Dr. Christopher Pilcher published in the September 2003 issue of the *Notebook*).

However, as Dr. Branson pointed out, using a positive HIV-RNA assay and a negative immunoassay as the standard by which to measure new HIV infections may only capture a small percentage of recently infected individuals. Generally speaking, HIV-RNA may be positive for only a few weeks before the more sensitive immunoassays—including rapid assays—detect antibodies to the virus. This, in turn, makes it difficult to capture enough people with recent HIV infection to render an incidence estimate with a reasonable confidence interval, especially when the incidence is less than 5% per year.

STARHS Search

TO TRACK THE INCIDENCE OF NEW HIV infections in the United States, the CDC has developed the Serologic Testing Algorithm for Recent HIV Seroconversions (STARHS), a sensitive/less sensitive HIV-antibody testing strategy to detect evidence of recent infections. STARHS involves conducting two EIAs on a single sample: a standard sensitive EIA and a "detuned" assay that is made less sensitive by using a higher serum dilution (1/20,000 compared to 1/400), a shorter specimen and incubation period, and a higher cut-off point to be considered positive. "The rationale here is that, as seroconversion progresses, antibody titers increase and antibody affinity increases," Dr. Branson explained. "If we have a sample that is nonreactive using the less-sensitive assay but reactive using the sensitive assay, we can conclude that the infection was recent and that seroconversion is still in its early stages. If, however, both the sensitive and less-sensitive assays are both reactive, we know that we're looking at someone who has been infected for a longer period of time."

The time from when a sample would first be reactive on a sensitive EIA to when the same sample would first be reactive on the less sensitive EIA is defined as the STARHS window period. The length of the window period is dependent on the cut-off used to distinguish a reactive from a nonreactive specimen in the less-sensitive EIA. Using a standardized optical density (SOD) cut-off of 1 for the bioMerieux Vironostika less sensitive EIA—the assay discussed by Dr. Branson—the mean STARHS window period has been determined to be approximately 170 days for purposes of deriving population-based HIV incidence estimates (see Figure 3).

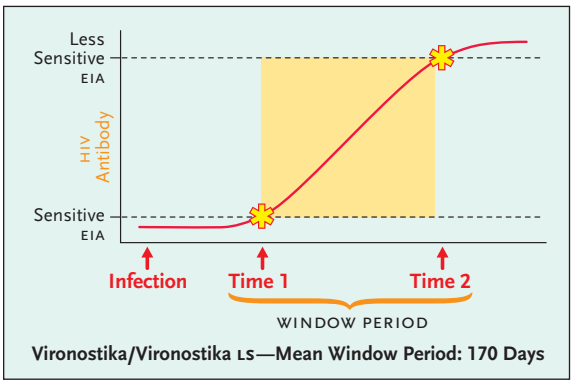

Figure 3. **STARHS and the Testing Window Period**

STARHS utilizes two EIAs: a standard EIA that is sensitive to low levels of HIV antibodies and a modified EIA that is less sensitive to low levels of HIV antibodies. At Time 1, the standard sensitive EIA will be reactive, because sufficient antibodies have been developed in order to detect HIV infection. At Time 2, the modified, less-sensitive EIA will be reactive, because total antibody levels are peaking. This indicates that infection is not recent. The time from when a person would first be reactive on a sensitive EIA to when they would first be reactive on the less sensitive EIA, if tested, is defined as the STARHS window period. The length of the window period is dependent on the less sensitive EIA cut-off used to distinguish a reactive from a nonreactive specimen. Using a standardized optical density cut-off of 1 for the bioMerieux Vironostika less-sensitive EIA, the mean STARHS window period has been determined to be approximately 170 days for purposes of deriving population-based HIV incidence estimates.

Source: U.S. Centers for Disease Control and Prevention

STARHS for Individual Results

IT'S IMPORTANT TO RECOGNIZE THAT INTERPRETING STARHS RESULTS FOR AN individual is complicated by two factors. First, there is significant variability in the time that antibodies develop in different seroconverters. Second, there is significant variability in the results of the less-sensitive assay.

As for the variability in the time that antibodies develop in different seroconverters, Dr. Branson pointed out that the SOD increases with time after seroconversion. The mean for the Vironostika-based study discussed above was 170 days. However, the window periods for different individuals ranged from 63 days to 404 days. "A considerable number of individuals have window periods greater than 170 days," he said.

In terms of the variability in test results, Dr. Branson explained that, compared with the sensitive assay, the less-sensitivity assay requires an extremely large dilution. "A very slight error when pipetting the specimen in the laboratory introduces a large error in the dilution," he said. "The timing and temperature for incubation must also be extremely precise. Practically speaking, it is difficult to achieve these stringent requirements, and so it is easy to introduce considerable error in the results from minor variations in the laboratory procedure."

In general, an SOD value less than 1 on the less-sensitive test can be interpreted to mean an individual probably seroconverted within the past year. According to Dr. Branson, "Interpreting an SOD value greater than or equal to one is not very satisfying. An individual with an SOD of one or greater may or may not have seroconverted more than one year ago. With only the STARHS results, it is not possible to say. Because of this, we are not necessarily recommending that people receive their results of these incidence tests. The results can be confusing to individuals being tested, especially when you try explaining that they may or may not have been infected within the past year."

Conclusion

THE CDC CONTINUES TO WORK WITH PROfessional medical associations and other partners to ensure that all health-care providers include HIV testing, when indicated, as part of routine medical care on the same voluntary basis as other diagnostic and screening tests. Previously, the CDC recommended that patients be offered HIV testing in high HIV-prevalence acute care hospitals and in clinical settings serving populations at increased risk (e.g., clinics that treat persons with STDs). The CDC has now strengthened this initiative in recommending offering HIV testing to all patients in all high HIV-prevalence clinical settings and to those with risks for HIV in low HIV-prevalence clinical settings. Because prevention counseling, although recommended for all persons at risk for HIV, should not be a barrier to testing, the CDC is now promoting adoption of simplified HIV-testing procedures in medical settings—such as POC rapid assays—with streamlined informed consent. To achieve this goal, the CDC continues to support state and local health departments in conducting demonstration projects offering HIV testing to all patients in high HIV-prevalence health-care settings and referral into care, treatment, and prevention services, and will assess the outcomes of these projects.

With respect to the detection of recent infections—an absolute must if the CDC and other health agencies are to have an impact on HIV transmission chains—efforts are underway to generate HIV incidence data, using STARHS, in various risk groups in the United States. This, Dr. Branson argues, should help to identify groups with the highest incidence, so that resources can be appropriately targeted, and the effectiveness of prevention efforts assessed. prn

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