

# State of the Art for Diagnosis of HIV Infection

**Bernard M. Branson**

Division of HIV/AIDS Prevention, National Center for HIV, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia

**Diagnostic tests for human immunodeficiency virus (HIV) infection have undergone considerable evolution since the first enzyme immunoassay (EIA) and Western blot were introduced 2 decades ago. Newer methods detect infection sooner and yield results much faster. Rapid tests represent a major advance for HIV screening in the United States. Six rapid tests for detection of HIV antibody have been approved by the Food and Drug Administration (FDA) since November 2002. Four of these tests can be done in point-of-care and nonclinical settings because they use whole blood or oral fluid and are simple to perform. An assay for detection of HIV-1 RNA has been approved by the FDA to detect HIV infection before seroconversion has occurred and to confirm results of reactive screening tests; pooled testing of specimens for HIV-1 RNA has increased the cost-effectiveness of this screening tool. These new testing technologies offer unique opportunities to diagnose HIV infection among the estimated 252,000–312,000 persons in the United States who are currently unaware they are infected.**

In 1985, the US Food and Drug Administration (FDA) licensed the first EIA to screen for HIV antibody, and shortly thereafter the Western blot was approved to confirm HIV infection. Since that time, numerous new tests have become available that are more sensitive and easier to perform. In addition, they produce results faster and detect infection earlier. Yet a survey by the Association of Public Health Laboratories revealed that, as of 2004, sixty-three percent of public health laboratories and many commercial laboratories were still using the Vironostika HIV-1 Microelisa System (Biomerieux), a test first introduced in 1987 that detects HIV later in the course of infection, compared with the newer tests [1].

The widespread adoption of the newer, rapid antibody tests is an essential component of the multifaceted strategy advanced by the Centers for Disease Control

and Prevention (CDC) to prevent HIV infection [2]. As more people are tested within the health care system, the facilities that provide the testing must be able to do it accurately and quickly. Also, it is often essential to give test results during the screening visit because many people do not return to receive results at a follow-up visit. The CDC estimates that, in 2000, fully 31% of persons who tested positive at publicly funded testing sites did not return to receive their test results [2]. Rapid HIV tests make it possible to perform the test in a matter of minutes, without the need for a return visit, and substantially increase the number of persons who receive their test results [3].

Although many clinicians may not know which specific assay will be used when they order an HIV test, the tests differ considerably. This article provides a brief review of the technological history of HIV test development; describes the new, rapid tests; and discusses issues, such as the choice of confirmatory test and the need for clinical judgment and follow-up, raised by use of the newer methods.

## **HIV TESTING: PATHOGENIC AND HISTORICAL PERSPECTIVES**

Studies show that viral transmission may be highest immediately after infection [4] and that persons who learn they are infected substantially reduce behaviors

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Reprints or correspondence: Dr. Bernard M. Branson, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Mailstop D21, Atlanta, GA 30333 (bbranson@cdc.gov).

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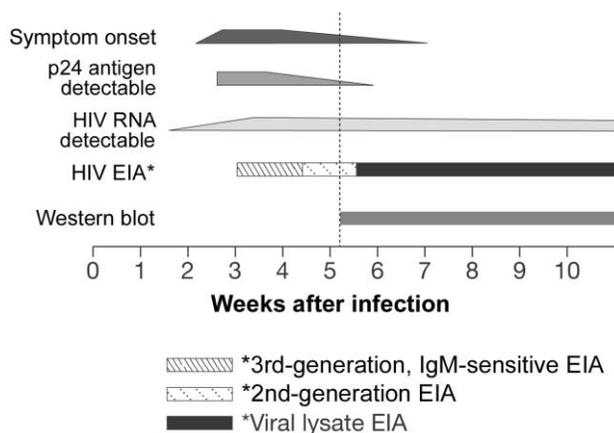
that are likely to transmit HIV [5]. Hence, tests that can help more people learn as soon as possible after exposure whether they are infected can do much to halt the spread of HIV.

EIAs, originally developed to screen blood for HIV, have evolved to detect HIV earlier during the course of infection. First-generation EIAs (e.g., Vironostika HIV-1 Microelisa System) and second-generation EIAs (e.g., Genetic Systems rLAV EIA; Bio-Rad Laboratories) detect IgG antibodies against HIV-1. Third-generation EIAs use “antigen sandwich” techniques that can also detect IgM antibodies against HIV-1, which develop earlier after infection. One, the HIVAB HIV-1/HIV-2 (rDNA) EIA (Abbott Laboratories), detects antibodies against HIV-1 and HIV-2; two EIAs, the Genetic Systems HIV-1/HIV-2 Plus O EIA (Bio-Rad Laboratories) and the Advia Centaur HIV 1/O/2 Enhanced (EHIV) EIA (Bayer HealthCare), also detect antibodies against HIV-1 group O. The latter, approved by the FDA in July 2006, uses a “random access” platform that allows the technician to select the HIV EIA from among 30 different assays loaded into the automated system and generates HIV test results in 58 min. Fourth-generation combination EIAs, widely used in other industrialized countries, identify HIV infection even earlier because they detect both HIV antibody and p24 antigen. Application for FDA approval of a fourth-generation EIA is anticipated in the near future.

Figure 1 depicts the time at which HIV infection can be detected by each of the different tests [6]. HIV infection remains undetectable until ~9 days after infection, after which nucleic acid amplification (NAAT) tests can detect HIV-1 RNA. The various generations of screening EIAs begin to detect HIV antibody 2–6 weeks later, sometimes before results of a Western blot (which is conventionally used for confirmation) are positive.

## RAPID HIV TESTS

Rapid HIV tests are single-use EIAs that contain all necessary reagents and yield results in  $\leq 30$  min. Because results of rapid tests are available quickly, persons can learn their test result during the same office visit and thus access effective treatment earlier, with significant benefits for long-term survival and quality of life. Rapid HIV tests are also essential when immediate results are necessary to make decisions about treatment. In one example, pregnant women whose HIV serostatus is unknown can be tested during labor, to determine whether antiviral treatment should be initiated to prevent mother-to-child transmission. In another example, an index patient can be tested after an occupational exposure, to allow prompt initiation of antiretroviral prophylaxis to the exposed health care worker [7, 8]. In high-volume, high-prevalence settings, such as emergency departments, rapid tests can make testing more feasible and generate results quickly enough to influence clinical management [9].



**Figure 1.** Time to HIV detection for various generations of diagnostic tests, relative to times of symptom onset and detection of p24 antigen and HIV RNA. Data are from [6].

**Tests recently approved by the FDA.** Six rapid tests have been approved by the FDA since 2002, all with sensitivities and specificities comparable to those of conventional EIAs (table 1) [10]. Four of the tests, when used with whole blood or oral fluid specimens, are designed for use in nonlaboratory settings. Two tests require serum or plasma samples and must be performed in a laboratory. As of December 2006, three of the rapid tests had received waivers under the Clinical Laboratory Improvement Amendments (CLIA). CLIA-waived tests must use unprocessed specimens, such as whole blood or oral fluid, and must be sufficiently simple to perform such that the chance of error is negligible. Receipt of a CLIA waiver enables point-of-care testing in settings that do not have dedicated laboratories. As with all screening assays, reactive rapid test results are preliminary and require confirmation with a supplemental test [11]. Rapid tests described here are listed in the order of their FDA approval.

The OraQuick Advance Rapid HIV-1/2 Antibody Test (OraSure Technologies) has received a CLIA waiver for use with an oral fluid sample or a whole blood specimen obtained via finger stick or venipuncture and can also be used with a plasma sample. The specimen is collected with a 5- $\mu$ L loop or by swabbing the upper and lower gums with the test device. The specimen and test device are added to a vial of developer solution, and test results are read 20–40 min later. If HIV antibodies are present, they bind to the peptides in the test location, causing a red line to appear. If the specimen was adequate, a red line also appears when IgG binds at the control location. A red line at only the control location indicates a valid, negative test. Red lines at both the control and test locations indicate a valid, positive test. When no line appears at the control location, the test is invalid and should be repeated [12].

The Reveal G3 Rapid HIV-1 Antibody Test (MedMira Lab-

**Table 1. Diagnostic characteristics of rapid HIV tests approved by the US Food and Drug Administration.**

Assay, by specimen analyzed	Sensitivity (95% CI)	Specificity (95% CI)
Whole blood		
OraQuick Advance Rapid HIV-1/2 Antibody Test	99.6 (98.5–99.9)	100 (99.7–100)
Uni-Gold Recombigen HIV test	100 (99.5–100)	99.7 (99.0–100)
Clearview HIV 1/2 Stat-Pak	99.7 (98.9–100)	99.9 (98.6–100)
Clearview Complete HIV 1/2	99.7 (98.9–100)	99.9 (98.6–100)
Serum or plasma		
Reveal G3 Rapid HIV-1 Antibody Test	99.8 (99.2–100)	99.9 (98.6–100)
Multispot HIV-1/HIV-2 Rapid Test	100 (99.9–100)	99.9 (99.8–100)

**NOTE.** Data are from [10].

oratories) requires a serum or plasma specimen; the 5-min procedure involves several steps to add the specimen, reagents, and buffer solution to the test cartridge, after which the test result is read immediately. This test is categorized as being of moderate complexity under CLIA and is generally performed in a clinical laboratory. Results are interpreted in the same manner as those of the other rapid tests: a red control line indicates a valid, negative test result. The presence of the control line and a red dot at the test location indicates a valid, positive test result [13].

Uni-Gold Recombigen HIV (Trinity Biotech) has received a CLIA waiver for use with whole blood specimens obtained via finger stick or venipuncture and can also be used with serum or plasma samples. It screens for HIV-1 and yields results in 10 min. A 40- $\mu$ L drop of blood, serum, or plasma is added to the specimen well on the test device, followed by 4 drops of buffer solution. Test results are also read visually from the test strip: a single red line in the control area means a valid, negative test result; 2 red lines indicate a valid, positive test result; and absence of a line in the control location means an invalid test [14].

Developed ~12 years ago, the Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad Laboratories) has been used widely outside of the United States but did not receive FDA approval until 2004. Although more complicated to perform than the other rapid tests, Multispot is approved by the FDA for distinguishing HIV-1 antibodies from HIV-2 antibodies. The test is categorized as being of moderate complexity under CLIA and requires fresh or frozen serum or plasma specimens. The multistep test procedure requires ~15 min to perform after the specimen has been prepared. In the presence of antibodies, a blue color develops at spots in specific locations on the cartridge membrane. Two spots indicate the presence of HIV-1 antibodies; a third indicates the presence of HIV-2 antibodies; and a fourth, the internal control, detects IgG [15].

Approved by the FDA in May 2006, the HIV 1/2 Stat-Pak Assay (Chembio Diagnostic Systems) received a CLIA waiver

in November 2006 for use with whole blood specimens obtained via finger stick or venipuncture [16]. The test can also be performed on serum or plasma. The specimen, obtained using a 5- $\mu$ L loop supplied with the test kit, is applied to the sample well on the test cartridge, followed by 3 drops of buffer. Valid results, indicated by the presence of 1 or 2 red lines, are read from the strip in 15–20 min. Of note, the test was renamed “Clearview HIV 1/2 Stat-Pak” in February 2007 [16].

The Sure Check HIV 1/2 Assay (Chembio Diagnostic Systems) was approved by the FDA in 2006 and detects HIV-1 and HIV-2. The manufacturer has applied for a CLIA waiver for use of the test with whole-blood specimens obtained by finger stick or venipuncture, but the test can also be performed with serum or plasma. The device resembles the barrel of a syringe, which houses the test strip. A 2.5- $\mu$ L specimen is obtained by capillary action into the tip of the barrel, which is then inserted into a vial of developer solution. Valid results, also indicated by the presence of 1 or 2 red lines on the white test strip, are read directly from the strip 15–20 min after the test is initiated. Of note, the test was renamed “Clearview Complete HIV 1/2” in February 2007 [17].

**Confirmation of and counseling patients about test results.**

Because rapid tests have sensitivities and specificities similar to those of conventional EIAs, a negative result of a rapid test is conclusive and generally requires no follow-up testing [18]. However, because results of these antibody tests may be negative before seroconversion, an individual with a possible recent exposure to HIV should be retested within 3 months. A reactive rapid test result, similar to a reactive result with a conventional EIA, requires further testing to confirm the diagnosis, usually with a Western blot or indirect immunofluorescence assay. If the confirmatory test result is negative or indeterminate, follow-up testing after 1 month is recommended, to rule out the possibilities of specimen mix-up or early infection that may not yet be detectable by Western blot [11].

Counseling persons who have reactive rapid tests is an important component of delivering test results. Counseling should

include information about HIV infection and treatment, measures that can be taken to avoid the potential transmission of HIV while awaiting results of confirmatory tests, and arrangements for further evaluation and treatment.

## RNA TESTS

Although antibody tests constitute the mainstay of HIV diagnosis, antibodies may be undetectable during the first 3–6 weeks after infection. RNA detection can play a valuable role in identifying early infection before seroconversion and for confirmation after reactive screening tests. In a retrospective study of archived plasma samples, an RNA test with sensitivity of 50 copies/mL detected HIV infection ~1 week before the p24 antigen test and 12 days before a sensitive, third-generation antibody test [6]. RNA NAAT testing has been used for quantifying HIV-1 loads and screening blood donations since 1999, but because the tests are expensive and technically complex to perform, they have not been adopted as widespread screening tools. New technologies and pooling strategies may change this situation.

To offset the cost of RNA testing, the state of North Carolina adopted an algorithm for pooled RNA screening of antibody-negative specimens obtained from individuals who participated in HIV counseling and testing programs [19]. With pooled testing, 100 antibody-negative specimens can be screened with just 1 RNA test by creating 10 miniature pools with 10 specimens each and then combining the 10 miniature pools into a master pool for screening. If the master pool is found to have  $\geq 1$  HIV-positive specimen, it is deconstructed to determine which of the miniature pools had  $\geq 1$  HIV-positive specimen, and the component specimens of that miniature pool are then tested to identify the positive specimen(s). In a study of 109,250 high-risk individuals screened with HIV antibody tests, 583 (0.5%) were antibody positive, and 23 (0.02%) who were antibody negative had acute HIV infection diagnosed on the basis of pooled RNA screening findings [19].

In September 2006, the Aptima HIV-1 RNA Qualitative Assay (Gen-Probe) was the first RNA NAAT test approved by the FDA for diagnosis of HIV infection. This assay uses transcription-mediated amplification, a NAAT technique currently used to screen urine or vaginal swabs specimens for chlamydial infection and gonorrhea, to detect HIV-1 RNA in plasma. The RNA is captured on magnetic microparticles and subsequently amplified for detection by means of a chemiluminescent probe. The procedure requires plasma specimens, includes several manual steps, and currently takes ~4.5 hours to perform [20]. The Aptima assay is approved as an aid to diagnosis of HIV-1 infection, including acute infection in patients without HIV antibodies, and as an additional test, when it is reactive, to confirm HIV-1 infection in an individual whose specimen is reactive for HIV antibodies.

It is estimated that 40%–90% of persons acutely infected with HIV will experience symptoms of acute retroviral infection, such as fever, lymphadenopathy, pharyngitis, and rash [21, 22]. For patients who experience a compatible clinical syndrome and who report recent high-risk behavior, health care professionals should consider a diagnosis of acute HIV infection. In these situations, tests for both plasma HIV RNA and HIV antibody should be performed [23]. Acute HIV infection is defined by detectable levels of HIV RNA in plasma from patients with negative or indeterminate results of HIV antibody tests. Although quantitative RNA (viral load) assays have been used for the diagnosis of acute HIV infection, they are not approved by the FDA for the diagnosis of HIV infection. A low-positive HIV RNA level (i.e., <5,000 copies/mL) may represent a false-positive test result, because RNA levels during acute infection are generally very high (i.e., >100,000 copies/mL) [24]. Patients with HIV infection diagnosed on the basis of HIV RNA testing should have serologic testing performed at a subsequent time to confirm that seroconversion has occurred.

## CONCLUSION

For nearly 20 years, first-generation and second-generation EIAs, with Western blot as the confirmatory test, constituted the gold standard for HIV testing in the United States. The availability of several fast, accurate, easy-to-use, and cost-effective HIV antibody tests will play an integral role in the CDC's initiative to encourage more-widespread HIV screening. Because many of the newer screening tests can now identify HIV-infected persons earlier during the course of infection than the Western blot can, a new algorithm will be necessary to confirm the presence (or absence) of infection. RNA assays might play an important role in confirmation, but that role is yet to be established because they also can give erroneous results.

The developments that make RNA testing more cost-effective might help identify HIV-infected people earlier during the course of infection, but this new generation of HIV tests must be ushered in thoughtfully. Contextual considerations are necessary to determine which test to use. RNA tests may detect infection earlier, but they offer little advantage if people do not learn their test results promptly. Easy-to-use rapid HIV antibody tests are feasible in a variety of clinical and nonclinical venues, but quality assurance must be maintained, particularly in nonlaboratory settings, to ensure that results are accurate. The nearly immediate results obtained with some of the newer tests mean that skilled professionals must be available to help HIV-infected people understand their illness and engage the medical care system.

We now have a growing technological armamentarium to help us identify HIV, but these are only tools. Clinical judgment and the clinician-patient relationship are essential to successful screening, accurate diagnosis, and effective follow-up.

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