

The Future of HIV Testing

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Abstract: HIV testing is the essential entry point for both treatment and prevention. The need to identify acute HIV infection (the period immediately after HIV acquisition, when persons are most infectious) and HIV-2 infection, which does not respond to many first-line antiretroviral agents, poses challenges for the traditional algorithm of Western blot confirmation after a repeatedly reactive antibody screening test. Immunoassays that detect antibodies earlier, tests for HIV RNA, and combination assays that screen simultaneously for both p24 antigen and HIV antibody are now approved for HIV diagnosis by the Food and Drug Administration. A revised testing algorithm can address the challenges posed by acute infection, HIV-2 infection, and the shortcomings of the Western blot. These new diagnostic strategies will allow earlier more accurate identification of infected persons so that they can benefit from effective treatment and also enhance abilities to focus prevention efforts where HIV transmission is most active.

Key Words: HIV antibody tests, acute HIV Infection, HIV diagnosis, HIV confirmatory tests

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Antibody tests have been essential to the diagnosis of HIV infection since the first HIV enzyme immunoassay (EIA) was introduced 25 years ago. Early concerns about false-positive test results in a setting of low prevalence¹ led to testing algorithms that emphasized specificity and the concept of “confirmatory” testing: positive HIV antibody test results require a repeatedly reactive screening test validated by a supplemental more specific test (such as the Western blot).² This testing algorithm served to establish the diagnosis of HIV for nearly 80% of the estimated 1.1 million infected persons in the United States.³ Identifying the estimated 21% of persons still unaware that they are infected and guiding effective future prevention efforts will require tests that detect HIV infection earlier, faster, and at less cost.

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EVOLUTION IN HIV TEST TECHNOLOGY

Figure 1 depicts the appearance of laboratory markers at different stages of HIV infection. As recently as 2006, nearly 70% of public health laboratories employed first-generation or second-generation assays that detect only immunoglobulin G antibodies to HIV-1, and only on average 45–60 days after infection.^{4,5} Thus, infection went undetected in many persons who were tested during the infectious seroconversion “window period.” During the 1990s, third-generation assays were developed that also detected immunoglobulin M (the first antibodies generated in the immune response) and did so an average 20–25 days after infection (Fig. 2).⁶ These tests also incorporated specific antigens to detect both HIV-1 and HIV-2. Because they were more expensive, third-generation assays were not widely adopted until the remaining first-generation HIV-1 assay was withdrawn from the market in 2007.⁷ The third-generation assays also complicate confirmatory testing because they become reactive before any bands appear on the Western blot.⁸ Although some clinicians order RNA viral load assays to diagnose early HIV infection, the first qualitative RNA assay was not approved by the Food and Drug Administration (FDA) for HIV diagnosis until 2006.⁹

EIAs are best suited for batch processing of large volumes of specimens in centralized laboratories. Because typical turnaround times for results range from a few days to more than a week, many infected persons failed to receive conventional test results.¹⁰ Since 2002, the FDA approved 6 rapid HIV antibody tests with sensitivities and specificities similar to those of first or second generation conventional EIAs.¹¹ Because these rapid assays can be performed in 30 minutes or less, their use allows many more patients to receive their test results.¹² Several factors, however, have begun to temper the initial enthusiasm for rapid tests. First, many persons who receive preliminary positive rapid test results do not return for their confirmatory test results and thus might not access necessary medical care.¹³ Second, reduced sensitivity during the early stages of infection contributes to false-negative results in some high-risk frequently tested populations in which rapid tests are often used. In one clinic, rapid tests detected infection in only 91% of antibody-positive men who have sex with men and in only 80% of those whose infection was documented by a combination of conventional antibody and RNA assays.¹⁴ Finally, rapid tests are impractical for large-scale screening programs in health care settings. Single-use rapid tests are time consuming to perform, and their cost remains persistently higher than that of conventional tests or the \$1–\$3 charged for identical tests outside the United States.

Since 2006, 2 random-access third-generation chemiluminescent immunoassays have received FDA approval.^{15,16} These run on automated platforms for a variety of tests in

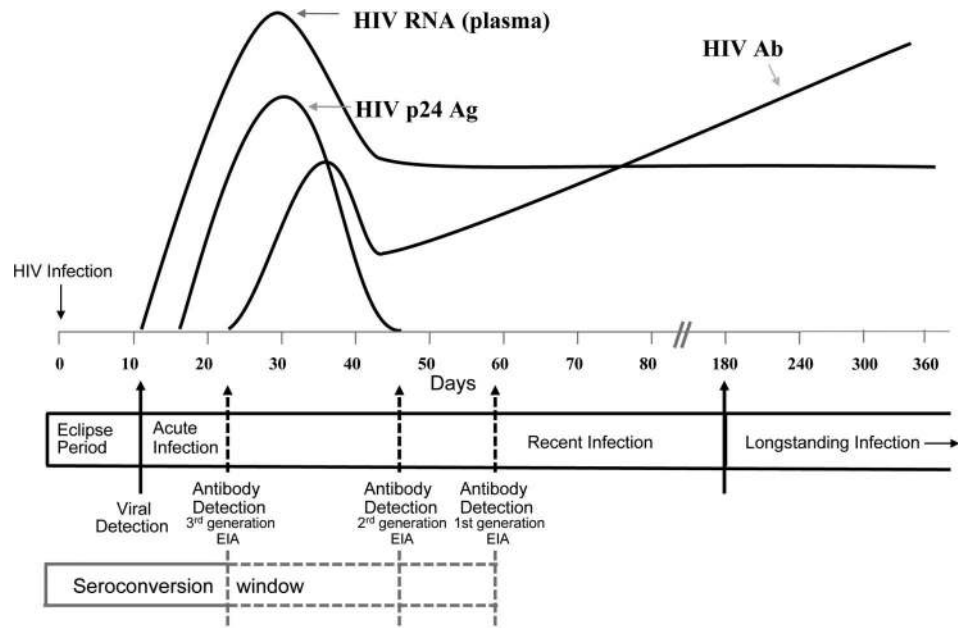


FIGURE 1. Sequence of appearance of laboratory markers in HIV infection.

addition to HIV, can test specimens individually or in batches, and generate test results in 1 hour or less. Random-access platforms are already widely available in many hospital and clinical laboratories and are well suited for screening programs that include HIV as one of the battery of tests ordered routinely for patients being seen in the emergency department or admitted to the hospital. Combination p24 antigen-HIV antibody (Ag/Ab) fourth-generation assays that identify $\geq 80\%$ of HIV infections otherwise detectable only by RNA have been used extensively worldwide for several years. The first fourth-generation Ag/Ab combination assay recently received FDA approval, and others are expected soon to become commercially available.¹⁷

CHALLENGES FOR HIV TESTING

Acute HIV infection (AHI) is defined as the interval between the appearance of HIV RNA and that of detectable antibodies (Fig. 1).¹⁸ Beginning with AHI, extremely high

levels of infectious virus are detectable in serum and genital secretions and persist for 10–12 weeks.¹⁹ Cohort studies suggest that the rate of transmission during AHI is 26 times as high as that during established HIV infection.²⁰ Mathematical models indicate that AHI, despite its short duration, can account for 10%–50% of all new HIV infections, especially in the context of high sexual partner concurrency or high rates of partner change.^{21–23} AHI screening programs that applied RNA assays to pooled antibody-negative specimens (to reduce per-patient costs) found that AHI generally represents only a small proportion (0.02% to 0.3%) of persons with negative HIV antibody tests but constitutes a substantial proportion (10%–25%) of new HIV diagnoses, especially among men who have sex with men.^{14,24–26} Until now, the high cost of RNA assays made routine screening for AHI impractical. Once they are commercially available, Ag/Ab assays that detect AHI 4–5 days later than RNA assays (Fig. 2) will allow widespread screening for AHI with an initial screening test.²⁷ Because most do not distinguish antigen from antibody reactivity, new testing algorithms will be required to distinguish AHI from established HIV infection.

Differentiating HIV-1 from HIV-2 poses another challenge. The number of HIV-2 diagnoses in the United States is believed to be low, but definitive diagnosis is difficult and surveillance is incomplete. Persons and partners of persons who acquired HIV-2 in West Africa have been diagnosed in Western Europe and the United States.²⁸ Because of cross-reactivity between HIV-1 and HIV-2 antigens, the HIV-1 Western blot may be interpreted as positive in patients with HIV-2.²⁹ “Cryptic” HIV-2 infection is thus often identified only after patients with an HIV-1 diagnosis manifest clinical deterioration despite a repeatedly undetectable HIV-1 viral load. HIV-2 has important implications for prognosis and treatment because HIV-2 does not respond to nonnucleoside reverse transcriptase inhibitors or to several protease inhibitors.³⁰

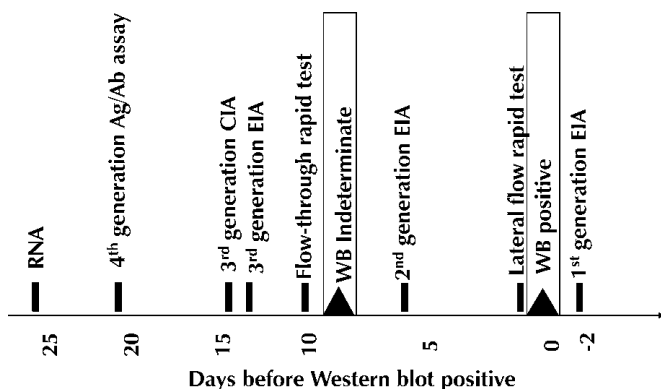


FIGURE 2. Reactivity of FDA-approved assays for HIV-1 compared with Western blot.

NEW STRATEGIES FOR HIV TESTING

Contemporary HIV testing strategies need to emphasize sensitivity, especially for the highly contagious phase immediately after infection. Despite longstanding concerns about false-positive test results, false-positive tests will be discovered and resolved promptly as part of subsequent testing for clinical evaluation. False-negative results, however, might not be detected for years, until HIV disease has advanced, after early effective treatment has been delayed, and after partners might have been unknowingly infected.

A revised testing algorithm has been proposed to address not only the challenges posed by AHI and HIV-2 but also the shortcomings of the Western blot.³¹ Testing begins with the most sensitive test possible, optimally a fourth-generation combination Ag/Ab test (Fig. 3). Repeatedly reactive specimens are then tested with an assay that differentiates HIV-1 from HIV-2 antibodies. Specimens that are repeatedly reactive on the Ag/Ab screening test but negative for antibodies are then tested for HIV-1 RNA. Detectable RNA establishes the diagnosis of AHI, which requires, in addition to linkage to medical care, urgent intervention to prevent further transmission and elicitation and evaluation of recent sex partners. In one study, persons with AHI named 2.5 times as many partners and nearly twice as many partners with undiagnosed HIV infection as did persons with longstanding HIV infection.³² However, the majority of HIV-infected persons will be antibody positive and can be immediately linked to medical care, where the recommended baseline clinical evaluation includes plasma HIV RNA (viral load).³³ If RNA is undetectable, further antibody testing (eg, Western blot) is indicated to determine whether HIV infection is present.

The frequency of AHI should be monitored to guide retesting recommendations. Both RNA and Ag/Ab tests reduce the window period after infection—they don't eliminate it. The 10-day duration of the eclipse period during which infection is undetectable (Fig. 1) is approximately the same as the interval during which AHI can be identified in antibody-negative persons. Therefore, the number of AHI cases might roughly approximate

the number of infected persons whose infection is undetectable. This suggests that persons seeking an HIV test after 1 or more recent risky exposures, especially in populations with an increased frequency of AHI, should be encouraged to retest in 3–4 weeks, even if their Ag/Ab test was negative. Evaluating factors associated with AHI can also be used to develop prediction models for persons at higher risk for HIV acquisition who need more frequent retesting and more intensive prevention interventions.³⁴ If it is not possible to screen with Ag/Ab tests (for example, in outreach settings when rapid HIV tests are used), retesting recommendations deserve particular attention. Individuals whose activities put them at higher risk of HIV acquisition and those from high-prevalence populations should be asked about recent potential exposures, multiple or concurrent sex partners, and other behaviors associated with increased HIV incidence (eg, methamphetamine use), and those with a higher likelihood of recent exposure should be encouraged to retest in 4–6 weeks.

HIV testing is the entry point for both care and prevention, and progress continues at a rapid pace. Rapid Ag/Ab combination tests and point-of-care tests for HIV RNA are in clinical trials. Promising techniques to determine whether antibody-positive persons were infected recently will soon help guide case finding and prevention and inform efforts to measure incidence. Because effective HIV treatment is available, doing everything possible to find infected persons and link them to care is more important than ever.

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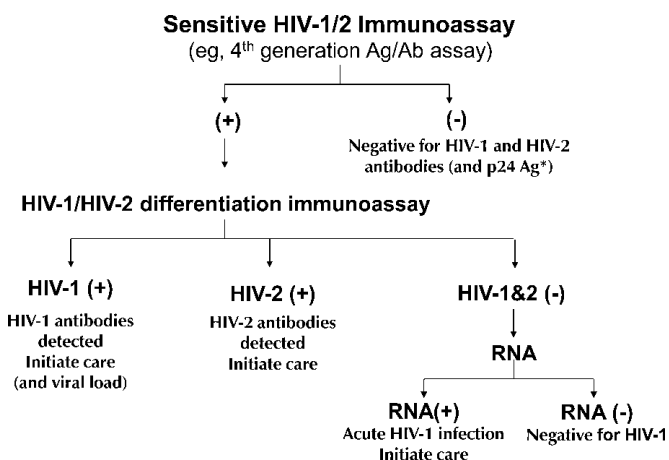


FIGURE 3. Proposed diagnostic algorithm for HIV diagnosis. *Denotes if Ag/Ab combo test is used.

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