Laboratory Diagnosis of HIV in Adults: A Review of Current Methods

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The estimated 1.2 million people infected with human immunodeficiency virus (HIV) in the United States, 20% are unaware of their diagnosis. Improved methods of HIV testing could decrease this number, as well as identify those who have recently acquired HIV infection and are at the most critical stage of infectivity. People with acute HIV infection have demonstrated enhanced transmission of HIV in multiple epidemiologic and pathogenetic studies. More than 50,000 HIV infections occur annually in the United States, and 30%–50% have been attributed to persons with recent infection. The original HIV diagnostic testing algorithm was developed by the Centers for Disease Control and Prevention in 1989. Recently proposed alterations to the algorithm would incorporate advancements made in HIV diagnostic testing, thereby increasing sensitivity while reducing turnaround time and cost. Improved diagnosis of acute HIV, and HIV type 2 in particular, would be expected. Knowledge of the available laboratory methods for HIV diagnosis is essential in the fight against the spread of HIV.

Keywords: human immunodeficiency virus; HIV; diagnosis.

Approximately 34 million people are infected with human immunodeficiency virus (HIV) worldwide, with 1.2 million of these residing in the United States. However, it has been estimated that 20% of HIV-infected individuals in the United States remain unaware of their diagnosis [1].

US HIV screening recommendations initially focused on testing individuals with a history of high-risk sexual behavior, sexually transmitted infections, blood transfusion between 1978 and 1985, or injection drug use [2]. However, in recent years, national efforts have been made to expand HIV testing further. In 2006, the Centers for Disease Control and Prevention (CDC) introduced the Expanded HIV Testing Initiative to broaden access to HIV screening and reduce the number of undiagnosed HIV infections. The CDC’s current recommendation is for routine HIV screening in all persons aged 13–64 years [3].

In resource-limited settings, the situation has proved even more challenging. In 2010, population surveys conducted by the World Health Organization found that >69% of individuals in 10 sub-Saharan African countries were unaware of their HIV status [4]. Efforts to increase awareness and screening for HIV have experienced limitations in the United States as well.

National HIV incidence surveillance data from the CDC estimates that >50,000 infections with HIV occur in the United States annually [5]. Ideally, expansion of testing for HIV would lead to not only more diagnoses but also more frequent detection of HIV during its acute stage. Acute HIV infection has been defined as both a transient nonspecific clinical syndrome associated with high viral replication and as the phase between appearance of detectable p24 or HIV RNA and detectable antibodies [6]. Studies have demonstrated that the highest rates of infectivity occur during the acute stage of HIV infection [7] and that enhanced transmission during this phase occurs regardless of viral load [8]. Estimates of symptomatic acute HIV vary, but according
PATHOGENESIS AND SIGNIFICANCE OF ACUTE HIV INFECTION

Plasma HIV RNA levels are not detectable during the eclipse period following HIV infection. They typically become detectable approximately 10 days after HIV infection, appearing several days before the p24 antigen (Figure 1). The rise in p24 antigen parallels the increase in viral load initially, peaking at about 3 weeks after infection then persisting for the next several months, while viral loads peak at about 3–6 weeks [10]. Subsequently, as the viral load declines, levels of HIV anti–p24 antibody and HIV anti–envelope antibody rise.

Risk of infectivity is closely correlated with HIV RNA levels. However, risk appears to be higher in acute than in established infection for multiple reasons. In acute HIV, the viral burden in plasma and genital secretions is particularly high [11]. It also has been reported that recently infected persons engage in more sexual activity than those with later stages of infection [12]. Higher infectivity in acute HIV is also thought to be due to the initial presence of homogenous variants more capable of causing infection as well as the absence of neutralizing antibodies present in chronic infection [13].

A study by Brenner et al found that persons with recent infections (ie, those infected <6 months following seroconversion) accounted for almost half of forward HIV transmission [7]. Other estimates of transmissions attributable to early HIV infection have been closer to 30% [14]. As a result, improved detection of recent HIV infection plays a critical role in decreasing the spread of HIV.

HIV DIAGNOSTIC TESTS

Enzyme Immunoassays

The first enzyme immunoassay (EIA) to detect HIV antibodies was introduced in 1985 [15]. These first-generation immunoassays detected immunoglobulin G (IgG) antibodies to HIV type 1 (HIV-1) using viral lysate as the antigen and were unable to detect antibody response to different HIV-1 clades [16]. They became positive approximately 6–8 weeks following infection [15]. First-generation assays lack the sensitivity and specificity of current widely used tests.

The second-generation assays increased specificity by using recombinant proteins or peptides to produce viral antigens. They were able to detect infection approximately 1 week earlier than the first-generation assays [15]. The development of the third-generation assays represented significant advancement as they could not only detect both HIV-1/2 immunoglobulin M (IgM) and IgG but also detect them as soon as 3 weeks after infection (Figure 1). They are also considered to be more sensitive than previous generation assays and by 2007, third-generation assays had supplanted earlier assays.

Fourth-generation assays became available in the United States in 2010, although similar combination assays have been utilized in other countries since a decade earlier. They simultaneously detect p24 antigen as well as HIV-1/2 IgG and IgM antibodies. With their ability to detect p24 antigen approximately 5–7 days after the appearance of nucleic acid [17], fourth-generation assays have succeeded in significantly shortening time to diagnosis to as early as 2 weeks after infection (Figure 1). Published data has confirmed that fourth-generation assays can establish HIV infection in >80% of individuals who tested positive by nucleic acid amplification test (NAAT) but either nonreactive or indeterminate by other assays [18–20].

HIV Confirmatory Tests

When screening immunoassay tests are repeatedly reactive, Western blot or indirect immunofluorescence assay (IFA) traditionally have been used as confirmatory tests due to their higher specificity. The Western blot assay tests for antibodies that bind to fixed HIV proteins, which when exposed to a substrate create a pattern which can be read as positive, negative, or indeterminate. The IFA, a less frequently used alternative, mixes serum or plasma samples with T cells expressing HIV antigens to check for presence of antibodies. Bound antibodies are then identified using an antihuman antibody conjugated to a fluorescent molecule.
The EIA/Western blot combination has demonstrated high sensitivity (99.3%–99.7%) and specificity (99.7%) once seroconversion occurs. However, due to their ability to detect only IgG antibodies [21], confirmatory tests can lag behind a reactive third- or fourth-generation assay by as much as 3 weeks, leading to false-negative results if the test is conducted before seroconversion. False-negative results are more likely in a high-prevalence population.

Quantitative viral load tests are not approved in the United States for diagnosis of HIV. In 2006, the Gen-Probe Aptima RNA qualitative assay was approved for diagnosis of HIV-1 infection as well as confirmation of a reactive EIA. Molecular RNA assays are highly sensitive in acute HIV; however, they can be negative in 3%–5% of samples with established infection [22, 23]. In addition, the currently available NAATs have several limitations, including skill, expense, and time necessary to perform these tests, as well as the necessity of blood draws. Technological research is now focused on simplifying NAATs and thereby making them more suitable for point-of-care testing [24] as well as decreasing the analytical time of real-time polymerase chain reaction (PCR) assays [25–27].

Rapid HIV Tests

Rapid HIV antibody tests have been Food and Drug Administration (FDA)--approved since 2002 and typically consist of lateral-flow devices or flow-through cassettes that use porous membranes to detect anti-HIV IgG and IgM in samples of oral fluid, whole blood, plasma, or serum. They offer the advantage of a turnaround time of 30 minutes or less, particularly important for populations with poor follow-up and women presenting in labor. Most detect both HIV-1 and HIV-2. Lateral flow tests do not require a laboratory and therefore can be performed in a variety of settings. Manufacturers have reported their sensitivities in the range of 99.3%–100% and specificities as 99.7%–99.9% [10]. However, various studies have reported lower sensitivities and specificities. Several studies have established performance characteristics comparable with first- and second-generation EIAs [28, 29], with a more recent study of the performance of 6 rapid HIV tests finding comparability to third-generation assays [30].

False-negative results, especially during the window period and in the higher-risk populations who often use rapid testing for HIV, remain a concern. One 2009 study of men who have sex with men showed that rapid tests were accurate in 91% of those with positive antibodies and in only 80% of those positive by a combination of antibody and RNA assay testing [31]. In addition, single-use rapid tests can be problematic for mass screening of populations due to their increased turnaround time and cost compared to conventional tests.

Oral fluid-based rapid tests are of particular interest in resource-limited settings and for use in home-based self-testing. They have been found to have comparable accuracy to blood tests and high accuracy in higher-risk populations, such as people attending sexually transmitted infection clinics [32]. Earlier this year, an FDA advisory panel universally endorsed the OraQuick In-home HIV test, which produces results in 20 minutes with a reported sensitivity of 93% and specificity of 99% compared to FDA-approved testing algorithms [33]. Such a test could prove very useful in HIV screening; however, the question of how best to ensure that self-testers get appropriate medical follow-up has yet to be settled.

A combination antigen (Ag)/antibody (Ab) rapid HIV assay (Alere DetermineHIV1/2Ag/Ab combo) has been developed but is not yet approved for use in the United States. However, available data indicate this assay cannot detect HIV p24 antigen at the same levels as laboratory-based fourth-generation assays and is unable to detect HIV infection as soon after infection as the laboratory assays [34, 35]. In addition to this commercial assay, there are several other rapid assays in the experimental pipeline that detect p24 antigen [36, 37]. Table 1 summarizes the currently available diagnostic tests for HIV infection.

FUTURE OF HIV DIAGNOSIS

The original HIV testing algorithm recommended confirming a repeatedly reactive HIV immunoassay with the more specific Western blot or IFA [38]. This algorithm was developed by the CDC in conjunction with the Association of Public Health Laboratories (APHL) in 1989 and has not been modified significantly despite interim advancements in HIV diagnostics. Yet the sensitivity and specificity of fourth-generation assays currently available for HIV screening have now surpassed those of the suggested confirmatory Western blot.

At the 2010 HIV Diagnostics meeting, the CDC and APHL proposed a new algorithm for the diagnosis of HIV. The proposed algorithm calls for using the most sensitive serologic HIV assay available (preferably a combination Ag/Ab assay) to screen for HIV. Use of an Ag/Ab assay would detect HIV earlier than third-generation immunoassays [17]. All reactive assays would be followed by a rapid IgG-antibody immunoassay that differentiates HIV-1 from HIV-2 and can act as a second specimen to confirm the initial screening assay. Confirmed reactivity would diagnose HIV. Specimens judged to be nonreactive in the second assay would be tested for HIV-1 RNA. If HIV-1 RNA is negative, further evaluation may be needed to determine if the patient is HIV negative or has acute HIV-2 infection. A figure comparing the proposed and current algorithms has been published elsewhere [39].

The proposed alternative algorithm would offer several advantages over the current algorithm. Using a rapid test for HIV confirmation rather than Western blot offers the advantages of
reduced cost and quicker turnaround time with more patients learning their diagnosis at time of testing. The Multispot HIV-1/HIV-2 Rapid (which is an example of the type of test recommended for confirmatory testing in the new algorithm but not yet FDA approved for this indication) can be read in 30 minutes [40]. The rapid test would also provide essential

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<td>Initial HIV tests</td>
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<tr>
<td>First- and second-generation immunoassays</td>
<td>Viral lysate (G1) or recombinant antigens (G2) capture anti-HIV Abs; specific IgGs detected using antihuman IgG</td>
<td>Detect HIV-specific IgG</td>
<td>Do not detect HIV-specific IgM and HIV antigens</td>
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<td>Third-generation immunoassays</td>
<td>Recombinant antigens capture anti-HIV Abs; IgG and IgM detected using antihuman Abs</td>
<td>Detect anti-HIV IgMs that may occur earlier in infection, in addition to IgG; improved seroconversion sensitivity; some have greater sensitivity in detecting HIV-2 and/or HIV-1 group O compared to earlier-generation assays</td>
<td>Do not detect HIV antigens</td>
</tr>
<tr>
<td>Fourth-generation immunoassays</td>
<td>Recombinant antigens capture anti-HIV Abs; IgG and IgM detected using antihuman Abs plus direct detection of p24 Ag</td>
<td>Detect Abs and Ags simultaneously, allowing recognition of HIV infection prior to seroconversion</td>
<td>May miss early HIV infection (prior to antigenemia)</td>
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<tr>
<td>Rapid tests</td>
<td>Immunoassays that employ lateral flow, immunoconcentration, or particle agglutination technologies</td>
<td>Completed in &lt;30 min often at point of care; performance characteristics similar to lab-based immunoassays (generation dependent)</td>
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<td>NAATs</td>
<td>Nucleic acids (DNA or RNA) amplified using specific primers and detected using labeled probes</td>
<td>Detect acute HIV infection prior to seroconversion</td>
<td>Most detect HIV-1 only; HIV-1 RNA may be undetectable in some Ab-positive HIV-infected persons; technically complex and expensive</td>
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<td>Supplemental HIV tests</td>
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<tr>
<td>Western blot</td>
<td>Viral lysate separated by electrophoresis, transferred to membrane and patient specimen is incubated with membrane to identify specific Ag/Ab complexes</td>
<td>High specificity due to Ag separation and concentration</td>
<td>Less sensitive than third- and fourth-generation immunoassays, technically complex, opportunities for technical error</td>
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<tr>
<td>Line immunoassays</td>
<td>Similar to WB, recombinant Ags or synthetic peptides replace viral lysate. Used outside of US</td>
<td>High specificity</td>
<td>Similar to WB</td>
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<td>Indirect immunofluorescence assays</td>
<td>Microscope slide coated with cells infected with HIV, patient specimen incubated on slide with cells and then fluorescently labeled antihuman Abs used to detect HIV specific Abs by microscopy</td>
<td>High specificity</td>
<td>Subjective interpretation of results; assays only approved for HIV-1 detection in US; expensive instrument (microscope) required; low throughput</td>
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<tr>
<td>NAATs</td>
<td>Nucleic acids (DNA or RNA) amplified using specific primers and detected using labeled probes</td>
<td>High specificity; detect HIV infection prior to seroconversion; may be used when WB is indeterminate</td>
<td>Most detect HIV-1 only; HIV-1 RNA may be undetectable in some Ab positive HIV-infected persons; technically complex and expensive</td>
</tr>
<tr>
<td>Enzyme immunoassays</td>
<td>Same as above for initial HIV tests</td>
<td>Same as above for initial tests. May distinguish between HIV-1 and HIV-2. More simple and rapid than WB and IFAs</td>
<td>None yet FDA approved for supplemental testing</td>
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Abbreviations: Ab, antibody; Ag, antigen; FDA, Food and Drug Administration; G1, first generation; G2, second generation; HIV, human immunodeficiency virus; IFA, immunofluorescence assay; IgG, immunoglobulin G; IgM, immunoglobulin M; NAATs, nucleic acid amplification tests; WB, Western blot.

Table 1. Summary of Diagnostic Tests for HIV Infection
Information on whether HIV-2 is present. HIV-1 Western blots can be misleading in patients with HIV-2 infection, as they may be positive due to cross-reactivity, or negative, potentially leading to a patient being mistakenly categorized as HIV negative [41]. Although HIV-2 remains extremely rare in the United States, and is restricted primarily to patients from West Africa, its identification has significance, given that HIV-2 may not respond to certain first-line antiretroviral drugs.

Testing for HIV-1 RNA can serve multiple purposes as well, including detecting acute HIV infection, distinguishing between acute and chronic HIV infection, and providing important information for further management. Of note, it may be necessary to collect a second specimen from the patient for RNA testing, as specimen storage conditions and handling techniques may differ from those employed for specimens used for serologic testing. Communication between the laboratory and clinician is essential to ensure appropriate specimens are collected to resolve these potential discrepancies.

Several studies published recently have supported the proposed algorithm. They have established that the proposed algorithm performs comparably to the current algorithm for diagnosis of HIV infection in individuals with established HIV infection [17, 42]. Among individuals with acute HIV infection, the proposed algorithm was significantly better at diagnosis than the current algorithm [17]. The proposed algorithm also produced fewer inconclusive results, and used fewer tests to diagnose HIV-2 than the current algorithm even when third-generation EIAs were utilized in both algorithms [39].

**DISCUSSION**

Great progress has been made in HIV management and outcomes over the past several decades. Worldwide efforts to increase access to both HIV care and screening have led to a decline of nearly 25% in the global rate of new infections between 2001 and 2009 [43]. However, approximately 7000 people continue to acquire HIV infection daily [43]. Rates of infection in the United States remain at approximately 50,000 per year with a notable 48% increase among young black men who have sex with men between 2006 and 2009 [7].

Delayed or missed diagnoses of HIV, particularly during the acute phase of infection, bear significant implications not only for the individual, but also in terms of the increased potential for spread of HIV as discussed earlier. Some studies have suggested that when people are aware of their diagnosis, they are more likely to change their sexual behavior [44, 45]. So although much progress in HIV diagnostic testing has been made, the benefits of further improvement are clear.

The initial HIV screening assay should be the most sensitive available test given the consequences of a missed diagnosis of HIV. All US labs should use fourth-generation screening tests instead of the third-generation EIAs still used in most US labs [42]. This change would increase the probability of diagnosing early infection with HIV and reduce transmission of HIV. In addition, widespread implementation of an updated algorithm for HIV diagnosis would utilize the most recent advancements in lab testing to increase diagnosis of acute HIV infection and of HIV-2.

Unfortunately, this algorithm would not be available in resource-limited settings. Despite significant global endeavors to reduce the incidence of HIV, HIV remains a worldwide epidemic that continues to affect poorer and less developed nations disproportionately. Accurate, accessible, and cost- and time-effective methods for diagnosis therefore remain a concern and an ever-evolving field for development and improvement. Low-cost, point-of-care tests have represented significant advancement, offering rapid results in a multitude of settings, from primary care clinics to resource-limited settings with weak healthcare infrastructure [46].

Even in resource-rich nations, appropriate application of HIV diagnostic testing presents numerous complexities. Although recommendations for universal screening exist, their efficacy has yet to be established. Future algorithms for HIV screening will also need to address appropriate scenarios for HIV retesting. While the proposed algorithm should prove more effective for screening the general population and detecting a higher percentage of acute HIV cases, some modifications would be appropriate when acute HIV infection is highly suspected.

If a provider suspects acute HIV, he or she could reasonably begin testing with both qualitative RNA reverse transcription (RT)–PCR and serology, particularly if third-generation assays remain widely utilized as the standard initial screening test. Official guidelines regarding subsequent retesting are not available and would be helpful in guiding the provider further. For example, if both tests are negative, RNA RT-PCR could be repeated in 2–4 weeks and serology could be rechecked in 3 months. Qualitative RNA RT-PCR could be negative in someone with chronic HIV infection or if tested too soon after risky exposure given that even RNA and Ag/Ab tests may not be positive in the first 10 days after infection. However, when high suspicion of acute HIV exists, retesting RNA RT-PCR is essential, given the risks of delaying diagnosis by several months. Clinical suspicion of HIV-2 also should lead to further diagnostic evaluations. If a patient is in the acute stage of HIV-2, the algorithm could miss the diagnosis and HIV-2 DNA PCR should be performed. Although HIV-2 proviral DNA PCR is available at some reference labs, it is not yet widely available.

Certainly, no algorithm can fit every scenario, and physicians will have to allow for individual circumstances and unique situations. However, if physicians fully understand the characteristics and limitations of available HIV diagnostic testing, they...
will have the basis for modifying the proposed algorithm most appropriately for each patient they encounter. In this way, each physician can contribute to the ongoing battle against the spread of HIV.

**Note**

**Potential conflicts of interest.** All authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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